Institut für Veterinärpathologie, Abteilung für Infektionspathologie
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Direktorin Institut: Prof. Dr. med. vet., Dipl.ECVP Anja Kipar
Leiterin Abteilung: Prof. Dr. med. vet., Dipl.ECVP, Nicole Borel

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Prof. Dr. med. vet., Dipl.ECVP, Nicole Borel

Elucidating the histologic findings of *Chlamydia pecorum*- induced arthritis in sheep

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Nina Ostfeld

Tierärztin
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Prof. Dr. med. vet., Dipl.ECVP, Nicole Borel, Referentin
Prof. Dr. med. vet. Michael Hässig, Korreferent

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Nina Ostfeld

Institut für Veterinärpathologie
ivpzdl@vetpath.uzh.ch

Elucidating the histologic findings of *Chlamydia pecorum*- induced arthritis in sheep

Chlamydia pecorum is an obligate intracellular pathogen with a wide host range such as sheep, cattle, goats and pigs as well as koalas. Chlamydial polyarthritis is the economically most important infection in young sheep. In the present study, tissues from experimentally and naturally *Chlamydia pecorum* infected sheep, were assessed histologically and immunohistochemically. Tissues of joints and inner organs of 35 sheep from different inoculation groups and of 5 naturally- infected lambs were investigated. Two different *C. pecorum* strains (IPA and E58), different routes of administration (intra-articular or intravenous), UVA- irradiated IPA strains, non-infected control groups and naturally infected sheep were compared. The most obvious inflammatory lesions were observed in joints and, interestingly, in the renal pelvis from both, experimentally and naturally infected animals. This resulted in chronic or chronic-active arthritis and pyelitis and intralesional chlamydial inclusions demonstrated by immunohistochemistry.

Immunohistochemical evaluation of the presence and distribution of macrophages, T and B cells in joints revealed macrophages. Previous observations indicated that *C. pecorum* isolates can infect circulating monocytes. Histological lesions in joints and inner organs and the presence of *C. pecorum* DNA, suggest chlamydial arthritis in lambs is the result of a systemic, hematogenous spread of *C. pecorum*.

Key Words: *Chlamydia pecorum*, arthritis, histopathology, immunohistochemistry, immune cells

Vetsuisse-Fakultät Universität Zürich (2020)

Nina Ostfeld

Institut für Veterinärpathologie
ivpzdl@vetpath.uzh.ch

Untersuchung histologischer Befunde in *Chlamydia pecorum* – induzierter Arthritis beim Schaf

Chlamydia pecorum ist ein obligat intrazelluläres Bakterium mit grossem Wirtsspektrum, einschliesslich Schafe, Rinder Ziegen, Schweine und Koalas. Durch Chlamydien verursachte Polyarthritis bei Lämmern ist von grosser wirtschaftlicher Bedeutung. In der vorliegenden Studie wurden Gewebeproben von Gelenken und inneren Organen experimentell und natürlich infizierter Tiere (n=40) histologisch und immunhistologisch untersucht. Es wurden zwei verschiedene *C. pecorum*- Stämme (IPA und E58), verschiedene Verabreichungsformen (intraartikulär und intravenös), UVA- bestrahlte IPA Stämme, die nicht infizierte Kontrollgruppe und natürlich infizierte Schafe miteinander verglichen. Die deutlichsten entzündlichen Veränderungen fanden sich in den Gelenken und Nierenbecken experimentell und natürlich infizierter Tiere. Dabei fanden sich chronische oder chronisch- active Arthritiden und Pyelitiden, sowie Nachweise von Chlamydien- Antigen mittels Immunhistochemie.

Die immunhistologische Untersuchung des Auftretens und der Verteilung von Entzündungszellen (Makrophagen, T- und B- Zellen) und deren Verteilungsmuster, zeigte ein deutlich gehäuftes Auftreten von Makrophagen. Frühere Studien belegen die Fähigkeit von *C. pecorum,* zirkulierende Monozyten zu infizieren. Die histologischen Veränderungen in Gelenken und inneren Organen sowie der

Nachweis von *C. pecorum* DNA, unterstreicht die Annahme einer systemischen, hämatogenen Streuung.

Schlüsselwörter: *Chlamydia pecorum,* Arthritis, Histopathologie, Immunzellen, Immunohistochemie

Elucidating the histologic findings of *Chlamydia pecorum*-induced arthritis in sheep

Ostfeld N.[1], Islam M.M.[2,3], Jelocnik M.[2], Hilbe M.[1], Sydler T.[1], Hartnack S.[4], Jacobson C.[5], Clune T.[5], Marsh I.[6], Sales N.[6], Polkinghorne A.[7,8], Borel N.[1]

[1]Institute for Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 268, 8057 Zurich, Switzerland

[2]University of The Sunshine Coast, Genecology Research Centre, University of the Sunshine Coast, Sippy Downs 4557, Australia

[3]Department of Pathology and Parasitology, Faculty of Veterinary and Animal Science, Hajee Mohammad Danesh Science and Technology University, Dinajpur-5200, Bangladesh

[4]Section of Epidemiology, Vetsuisse Faculty University of Zurich, Winterthurerstrasse 270, 8057 Zurich, Switzerland

[5]College of Science, Health, Engineering and Education, Murdoch University, Perth, Western Australia 6150, Australia

[6]NSW Department of Primary Industries, Elizabeth Macarthur Agricultural Institut, Menangle, New South Wales 2568, Australia

[7]Department of Microbiology and Infectious Diseases, Nepean Hospital, NSW Health Pathology, Penrith 2750, New South Wales, Australia

[8]Nepean Clinical School, University of Sydney Medical School, University of Sydney, Penrith 2750, New South Wales Australian

Corresponding author: Prof. Dr. Dipl. ECVP Nicole Borel, Institute of Veterinary Pathology, Vetsuisse Faculty, University of Zurich, Winterthurerstrasse 268, CH-8057 Zurich, Switzerland. Email: N.Borel@access.uzh.ch. Telephone: 0041446358563

Abstract

Chlamydia pecorum is an obligate intracellular pathogen with a wide host range including livestock such as sheep, cattle, goats and pigs as well as wildlife species such as koalas. Chlamydial polyarthritis is the economically most important infection resulting in swollen joints, lameness, stiffness and weight loss in young sheep. In the present study, tissues from either *Chlamydia pecorum* experimentally or naturally infected sheep, were assessed histologically and immunohistochemically. In total, 312 tissues of carpal, hock and stifle joints as well as spleen, liver, kidney, lymph nodes, lung, conjunctiva and brain of 35 sheep from different inoculation groups and of naturally- infected lambs were investigated. Two different *C. pecorum* strains (IPA and E58), different routes of administration (intra-articular or intravenous), UVA-irradiated IPA strains, non-infected control groups and naturally infected sheep were compared. The most obvious inflammatory lesions were observed in joints and, interestingly, in the renal pelvis from the experimentally infected group and naturally infected animals. This resulted in chronic or chronic-active arthritis and pyelitis and intralesional chlamydial inclusions demonstrated by immunohistochemistry.

Immunohistochemical evaluation of the presence and distribution of macrophages, T and B cells in joints revealed macrophages as the most prevalent inflammatory cell population. Previous observations indicated that *C. pecorum* isolates can infect circulating monocytes. Together with the finding of the histological lesions in joints and inner organs alongside the presence of *C. pecorum* DNA, these observations suggest chlamydial arthritis in lambs is the result of a systemic, hematogenous spread of *C. pecorum*.

Key Words: *Chlamydia pecorum*, arthritis, infection model, lambs, histopathology,

immunohistochemistry, immune cells

Introduction

Chlamydia (C.) pecorum is a gram-negative, obligate intracellular bacterium with a broad host range including livestock such as sheep, cattle, goats and pigs as well as wildlife species like koalas. [7,17,52] Infection with *C. pecorum* can present with clinical manifestations such as arthritis, conjunctivitis, infertility, enteritis, endometritis, vaginitis, pneumonia, mastitis as well as sporadic bovine encephalomyelitis (SBE) in cattle. [23,37,43,50,51] The most important economical manifestation of *C. pecorum*, mainly prevalent in Australia and New Zealand, is polyarthritis in sheep which causes swollen joints, lameness, stiffness and weight loss in lambs. [50] Moreover, it is hypothesized, that the agent also infects the gastrointestinal tract of lambs asymptomatically.[13] In Australian sheep flocks, prevalence of gastrointestinal shedding of >30% has been reported.[53]

Recent studies have used comparative genomics and molecular typing to identify differences in strain virulence.[23,25,1,41] It was shown that polyarthritic sheep had unique sequence types (e.g. ST23) when compared to rectally shed *C. pecorum* strains (e.g. ST62, ST63, ST81).[1]

The research group of Storz described polyarthritis in feeder lambs due to a PLGV agent (psittacosis-lymphogranuloma venerum agent) in the USA in the 1960´s.[46,47] The agent induced inflammatory changes in synovial membranes and had the ability to pass the synovia/blood barrier followed by entry into the blood stream and causing mild inflammatory reactions in internal organs. Experimentally infected sheep showed identical pathological changes to those observed in naturally infected sheep recovering from PLGV-induced polyarthritis.[46] These early infection studies were

recently reproduced by Australian researchers.[22] While these experimental studies [46,47,22] focussed on clinical signs and gross findings due to *C. pecorum* infection in lambs, limited information is available on the histopathomorphological changes in infected animals. Therefore, the aim of our study was to examine joints and inner organs histologically and immunohistochemically by using specific quantitative and semiquantitative scoring systems. The use of such scoring systems might standardize and better characterize the pathogenesis of *C. pecorum*-induced arthritis in sheep and other arthritic diseases.

Materials and Methods

Ethics statement

This study was carried out in accordance with the New South Wales (NSW) Animal Research Act (1985) and Animal Research Regulation (2010); Australian Code for the Care and Use of Animals for Scientific Purposes. The protocol was considered and approved by the University of the Sunshine Coast Animal Ethics Committee (AN/S/18/56) and the Elizabeth Macarthur Agricultural Institute (EMAI) Animal Ethics Committee (M17/09). Animals were housed in Quarantine Containment Level 2 facilities at the Elizabeth Macarthur Agricultural Institute (EMAI) and veterinary procedures were carried out by qualified veterinarians. Naturally infected sheep were part of a project approved and monitored by Murdoch University Animal Ethics Committee (R3004/17).

173 *Experimental setup and groups, sample collection, histologic and PCR examination*

174

175 The experimental design was implemented during a previous study [22] and consisted
176 of 35 Australian Merino sheep, 6 - 7 months of age and originating from a flock
177 without any history of arthritis or *Chlamydia*-related health problems. These 35
178 sheep were selected following two rounds of prescreening for *Chlamydia* in over 100
179 sheep, collecting rectal swabs for a *C. pecorum*-specific qPCR as well as blood
180 samples for complement fixation test (CFT).[4] Both tests confirmed *Chlamydia*-
181 negative status in the 35 animals selected for experimental studies. All 35 sheep
182 were weighed and randomly assigned to four groups: 1) IPA-group (n=10), 2) E58-
183 group (n=10), 3) UV-irradiated IPA-group (n=10) and 4) control group (n=5). The
184 infection groups received 10^7 inclusion forming units (IFU) of *C. pecorum* E58 or IPA
185 strain either into the right carpal joint via the intra-articular route (IA; n=5 per group)
186 or intravenously (IV; n=5 per group). The control group (n=5) received Sucrose
187 Phosphate Glutamate (SPG) by the IA route (n=5). The *C. pecorum* IPA isolate used
188 in this study originated from the joint of a sheep suffering from polyarthritis in the
189 USA, while the E58 is also a USA isolate but was retrieved from the brain of a calf
190 suffering from sporadic bovine encephalomyelitis.[33]
191 Euthanasia, necropsy and tissue sampling were performed at various time points
192 (Table 1) after the administration of the inoculum as described previously.[22] Tissues
193 of carpal, hock and stiftle joints and from the following inner organs: kidney, liver,
194 spleen, lungs, lymph nodes and brains from experimentally infected animals were
195 collected in 10% formalin and processed as previously described.[22]

196 In an additional study, five sheep (3619, 7578, 7629, 8084, 8452), approximately six
197 months of age, from a commercial farm approximately 165 km south-east of Perth,

198 Australia, were assessed as having swollen joints and chronic lamenness. One
199 sheep also had evidence of conjunctivitis at the time of examination. Sheep were
200 euthanized via intravenous barbituate overdose. Tissues of left and right carpal joint,
201 liver, kidney, mesenteric lymph node and lung were collected from these naturally
202 infected animals, fixed using 10% formalin and processed as previously described.[22]
203 Samples of spleen were collected for all sheep except number 8084. In addition,
204 tissues of the right stifle joint, the left conjunctiva and third eyelid from animal
205 number 3619 were available. Sterile dry swabs were used to sample synovia
206 (carpus) and screened for *Chlamydia* with the previously described *C. pecorum*-
207 specific qPCR. [26] This confirmed the presence of *C. pecorum* DNA in joints (synovia)
208 for all five sheep (5/5).

209 In total, 110 formalin-fixed and paraffin-embedded (FFPE) tissue blocks deriving
210 from experimentally inoculated sheep (n=35) and naturally infected animals (n=5)
211 were included in this study. Hematoxylin and eosin (HE) stained slides were
212 prepared from all tissues according to standard procedures.

213 To detect chlamydial DNA, qPCR was performed on the joint tissues of both
214 experimentally and naturally infected animals and the organs of only the
215 experimentally infected animals. Positive qPCR results were listed per group, organ
216 or joint and were compared against each other considering copies per microliter.

217

218 *Joints*

219 From experimentally infected animals, a total of 32 slides from carpal, hock and stifle
220 joints were examined (Supplemental Table S1). From naturally infected animals
221 (n=5), a total of 12 slides from carpal, hock and stifle joints (10/12 carpal joints and

2/12 stifle joints) were investigated (Supplemental Table S2). The assessment was performed by two pathologists in a blinded manner (at a magnification of 100x and 400x) by using a light microscope (Leitz, Laborlux S, Germany) and pathological diagnoses were elaborated. Five main microscopic findings, focussing on inflammation, were identified and assessed qualitatively: The presence of inflammation, the type of inflammation, the degree and distribution of inflammation and whether the surrounding tissue was affected or not (Fig. 1). Then, inflammatory joint lesions were evaluated according to semi-quantitative parameters resulting in a newly developed scoring system with a maximum reachable score of 48 per animal (Table 2). In addition to parameters described in Fig. 1, the scoring system also included important findings such as the presence of fibrin and granulation tissue and positive immunolabeling for *Chlamydiaceae*. Furthermore, the synovia was evaluated according to criterias as previously described [10,15,28] and total scores per experimental group were evaluated (Table 3).

Inner organs

From experimentally infected animals, a total of 72 slides from inner organs (livers, kidneys, brains, spleen, lymph nodes and lungs) were examined. From naturally infected animals (n=5), a total of five slides were examined from inner organs while only 1/5 conjunctival tissues were investigated. The assessment was performed by two pathologists in a blinded manner (at a magnification of 100x and 400x) by using a light microscope (Leitz, Laborlux S, Germany) and pathological diagnoses were elaborated (Supplemental Table S3). The four main microscopic findings of inflammation were identified and assessed qualitatively: these were the presence of inflammation, the type, degree and the distribution of inflammation (Fig. 2).

Inflammatory lesions of inner organs were also evaluated according to semi-quantitative parameters, resulting in a newly developed scoring system with a maximum reachable score of 39 per animal (Table 4). In addition to the parameters described in Fig. 2, the scoring system also included the prescence of positive immunolabeling for *Chlamydiaceae*.

For lung tissues, high-resolution digital scans were acquired using the Hamamatsu Photonics's NanoZoomer HT2.0 (Hamamatsu, Japan). All bronchi and bronchioli per lung section were measured and for those with a minimal diameter of 200 micrometer, peribronchial and peribronchiolar lymph follicles (representing BALT-hyperplasia) and other lymphocytic accumulations were assessed.

Lymph nodes and spleen were analyzed for the presence of hyperplasia as previously described.[3] The area of the whole tissue on each section was measured, the number of follicles were counted at a magnification of 100x and assessed semiquantitatively using a newly developed scoring system (0 = 10, 1 = >20, 2 = >50, lymph follicles per section and 0 = 1-4, 1 = 5-10, 2 = >10 secondary follicles with germinal centers). For lymph nodes and spleen, a minimum of five randomly chosen slides per each group was examined in a blinded manner. All tissue assessments were performed according to the targeted masked review, as previously described.[31]

Immunohistochemistry

Immunohistochemistry (IHC) to detect chlamydial antigen in tissues of joints and inner organs of experimentally infected, naturally infected and control animals was performed as previously described.[22] A total of 248 IHC sections were examined using a light microscope (Leitz, Laborlux S, Germany) at a magnification of 100x and

400x. A positive immunohistochemistry signal was included into the scoring system (Supplemental Table S1, S2 and S3, Table 2 and 4). IHC was considered positive if an intracytoplasmic granular labeling in at least one cell (organ-specific) was present.

FFPE joint sections (n=45) were re-evaluated for the presence and percentage of inflammatory cells (macrophages, B and T cells) by immunohistochemical labeling to detect Iba1 (macrophages), CD20 (B cells) and CD3 (T cells) antigens. Briefly, the sections were mounted on positively charged slides and dried overnight at 37˚C. After deparaffinization, different antigen demasking protocols for each antibody were applied (Table 5). For the semi-quantitative evaluation, areas with inflammatory infiltrations were first selected in HE stained slides at 100x magnification. Then, the percentage of IHC-positive labelled cells (ranging from 0-100%, in 5 % steps) was counted in five randomly selected regions in corresponding IHC-slides for Iba1, CD20 and CD3 at 400x magnification.

Statistical analysis

Statistical analyses were performed on data from experimentally infected animals and the corresponding control group using the software R [40] version R3.6.3. Generalized mixed effect models with a poisson distribution were performed using the lme4 package to evaluate the results of histological assessement of joint and inner organ lesions using the newly developed scoring system.[2] Because liver lesions were only mild and also present in control and UV-irradiated groups, we can assume that they were not related to the *C. pecorum* infection and were therefore excluded from the statistical evaluation. As fixed effects, group and joints or organs,

and as random effects, animals were considered. To adjust for multiple comparisons, Dunnett's or Tukey's approach containing the package multcomp [18] were chosen.

In lung tissues, bronchioli with a minimal diameter of 200 micrometer were assessed for peribronchiolar lymphocytic accumulation and lymph follicles (representing BALT-hyperplasia). Lymph nodes and spleen were analyzed for the presence of hyperplasia by the assessment of lymph follicles as well as secondary follicles using a newly developed scoring system in consideration of the area-size of each tissue section. Linear models were used for the evaluation of lung tissues and tissues of spleen and lymph nodes. The measurements of the biomarkers (CD3, CD20, Iba1) were dichotomized into absence and presence and the proportion of present biomarkers was described with Wilson's binomial 95% confidence intervals using the package, Desctools.[45] Generalized estimation equations were used to assess if the biomarkers differed between the experimental groups and locations by using the package, geepack.[19] The qPCR measurements in the joints and organs were analyzed with mixed poisson and zero-inflated poisson models using the packages lme4 [2] and glmmTMB.[8]

Results

Histologic findings in joints

Joints of animals from control and IPA UV-irradiated groups did not show any signs of inflammation. In joints of experimentally infected animals, the majority of inflammatory lesions were chronic (lymphoplasmahistiocytic including granulation tissue) (Fig. 3, 4), followed by chronic-active (lymphoplasmahistiocytic and purulent including fibrin accumulations) arthritis/synovitis (Fig. 5, 6). Six of the evaluated joints

(n=94) showed an acute arthritis/synovitis. According to the semi-quantitative parameters in the scoring system, the most severe histological joint lesions were observed in the IPA i.v. group, followed by the E58 i.a. group. Moderate inflammatory changes were seen in the IPA i.a. group, followed by mild lesions in the joints of the E58 i.v. group (Table 6).

Additional findings (synoviocyte proliferation, neovascularisation and periarterial fibrosis) (Table 3) were evaluated and the IPA i.v. group had the highest number of animals (14/20) which showed one or more of these pathological lesions (Fig. 7, 8). Stifle joints had the highest score followed by carpal and hock joints (Supplemental Table S4). An investigation of joint pathology score differences between the experimental groups and the control group were analyzed using generalized mixed effect models with a poisson distribution. The groups E58 i.a., IPA i.v. and IPA i.a. differed significantly from the control group (Supplemental Table S5).

In joints of naturally infected animals, 5/5 animals had inflammatory lesions. The majority of inflammatory lesions were chronic-active processes (lymphoplasmahistiocytic and purulent arthritis/synovitis) (Fig. 9, 10). The right carpus of animal number 7629 did not show any inflammatory signs.

Histologic findings in inner organs

The brains of animals from the control and IPA UV-irradiated groups did not show any signs of inflammation. In brains of the experimentally infected animals, the most obvious inflammatory lesion was a mild chronic lymphohistiocytic (meningo-) encephalitis (5/20 animals) (Table 7, Fig. 11). In 3/35 brains (UVA-IPA i.a.: n=1, E58 i.v.: n=1, IPA i.v.: n=1), glia nodules were present (Fig. 11, insert). In these cases, by examination of brain sections following immunohistochemistry, a toxoplasmosis

infection was assessed (immunohistochemistry for detection of *T. gondii* using the rabbit polyclonal antibody RB-282-A0, Thermo Fisher Scientific; dilution 1:50) with negative results.

Lung tissues from all experimental animals (n=35) did not reveal any signs of inflammation, thus no significant differences were found between the groups. BALT hyperplasia was present in 23/35 lungs in animals from all groups (Fig. 12). Secondary follicles were found in 28/35 lymph nodes in animals from all groups (Fig. 13).

A mild chronic multifocal periportal hepatitis was present in 5/15 livers of animals from the control and the IPA UV-irradiated groups (Fig. 14). Similar lesions were also observed in 8/20 livers of animals in the other experimentally infected groups. Occasional leukocytostasis in liver vessels was present in all groups (Table 7).

The control and IPA UV-irradiated groups did not exhibit any lesions in the kidneys (Table 7). In other groups of infected animals, the most obvious kidney lesion was a chronic or chronic-active pyelitis, which partially ascended into the interstitium and was most severe in the IPA i.v. group (Fig. 15, 16). Statistical analyses of differences between the experimental groups compared to the control group revealed significant differences in group IPA i.v. (Supplemental Table S5).

Tissues of spleen and lymph nodes from all experimentally infected animals (n=35) did not reveal any signs of inflammation and no significant differences were found (Supplemental Table S4). For the investigation of differences between the experimental groups compared to the control group in lymph nodes, linear model measurement was performed and revealed a significant difference ($p< 0.08$) between numbers of follicles in the E58 i.v. group compared to the control group but

no significant differences regarding the numbers of secondary follicles (Supplemental Table S5).

In naturally infected animals, lungs, lymph nodes and spleen did not show any inflammatory lesions. A mild chronic multifocal periportal hepatitis was present in livers of all animals (n=5). In 5/5 animals, a chronic (animals number 3619 and 7629) or chronic-active (animals number 8452, 7578 and 8084) pyelitis, which partially ascended into the interstitium, was seen (Fig. 17). A severe chronic (lymphoplasmahistiocytic) multifocal conjunctivitis was seen in animal number 3619 (Fig. 18).

Immunohistochemistry

Immunohistochemical labeling for detecting chlamydial antigen in experimentally infected animals revealed positive results in the carpal joint of animal 628 from the IPA i.a. group, in the stifle joint of animal 658 from the IPA i.v. group, in carpal, hock and stifle joint of animal 710 from the E58 i.a. group and in the kidney of animal 616 from the IPA i.v. group (Fig. 19, 20).

In naturally infected animals, immunohistochemical labeling for detecting chlamydial antigen revealed positive results in the left and right carpal joint of animal number 3619 (Fig. 21), in the right carpal joint and kidney of animal 7578 (Fig. 22), the left carpal joint of animal number 7629, the right carpal joint of animal number 8084 and the kidney of animal number 8452.

Immunohistochemical labeling in experimentally infected animals to detect Iba1 (macrophages, Fig. 23), CD20 (B cells, Fig. 24) and CD3 (T cells, Fig. 25) antigens was histologically most obvious for detecting macrophages (iba1). The same pattern

was observed in the five naturally infected animals using antibodies against Iba1 (Fig. 26), CD20 (Fig. 27) and CD3 (Fig. 28).

For the immunohistological labeling (Iba1, CD20, CD3) in experimentally infected animals, a statistical evaluation was performed. Wilson's binomial 95% confidence intervals revealed the highest proportions in positive stained cells for Iba1 labeling (7.39), followed by CD20 (3.12) and CD3 (1.80) with the highest amount in carpal joints (170), followed by stifle (160) and hock joints (155). For CD3 labeling, the groups IPA i.a., IPA i.v. and E58 i.a. differed significantly from the control group with the highest presence in carpal joints followed by stifle and hock joints (Supplemental Table S5, Fig. 29). CD20 labeling revealed a significant difference in the E58 i.a., IPA i.a. and IPA i.v. group when compared to the control group with the highest presence in carpal joints followed by stifle and hock joints (Supplemental Table S5, Fig. 30). For Iba1 labeling, the groups E58 i.a., IPA i.v. and IPA i.a. group differed significantly from the control group with the highest presence in carpal joints followed by stifle and hock joints (Supplemental Table S5, Fig. 31).

qPCR results

By qPCR, 8/15 joint tissues from the IPA i.a. group were positive for chlamydial DNA (5/5 carpal joints, 1/5 hock joint and 2/5 stifle joints) with a range of 71 to 142'313 copies/µl. In the IPA i.v. group, 11/15 joint tissues were tested positive for chlamydial DNA (qPCR, 2/5 carpal joints, 5/5 hock joint and 4/5 stifle joints) with a range of 130 to 2506 copies/µl. In the E58 i.a. group, 5/15 tissues of carpal joints were positive for chlamydial DNA via qPCR with a range of 9641 to 167'349 copies/µl and in the E58 i.v. group, 8/15 joint tissues were positive (qPCR, 2/5 carpal joints, 4/5 hock joint and 2/5 stifle joints) with a range of 92 to1884 copies/µl. Wilson's binomial 95%

confidence intervals revealed the highest proportions in carpal joints followed by stifle and hock joints (Supplemental Table S4). This was also confirmed by mixed poisson and zero-inflated poisson models. The groups which received intra-articular inoculation differed significantly from the intravenously inoculated groups (Supplemental Table S5). In carpal, hock and stifle joints, the highest qPCR loads (copies/µl) were evaluated in the IPA i.a. group (Supplemental Table S4, loads per joints and groups). Regarding the inner organs, Wilson's binomial 95% confidence intervals revealed the highest proportions of qPCR loads in lymph nodes, followed by liver, brain, kidney, spleen and lung (Supplemental Table S4). Mixed poisson and zero-inflated poisson models revealed a significant difference in the qPCR load of the lungs. The analysis revealed no significant differences between the groups (Supplemental Table S5). In brains, kidneys, livers and lungs, maximal qPCR loads (copies/µl) were evaluated in the E58 i.v. and in the IPA i.v. groups in lymph nodes (Supplemental Table S4, loads per organs and groups).

In naturally infected animals, 4/5 joint tissues (left hock of animal number 3619, left carpus of animal number 7629, right carpus of animal number 7579 and right carpus of animal number 3619) were positive for chlamydial DNA with a range of 6.76 to 163 copies/µl.

Discussion

Chlamydia pecorum-associated arthritis in sheep is an economically important infectious disease in Australia causing losses to the sheep industry.[50] In this study, we investigated histomorphological lesions of joints and inner organs from experimentally and naturally infected sheep to elucidate the pathogenesis of *C. pecorum*-associated arthritis. Tissue samples were retrieved from an experimental

challenge model in sheep. [22] This challenge model evaluated two different inoculation routes (intraarticular, intravenous) and the disease progression of two virulent *C. pecorum* isolates (E58, IPA). The challenge study focussed on clinical outcomes and gross pathology lesions with the aim of establishing an infection model that can be utilized in the future for potential vaccine validation. *C. pecorum* E58 was isolated from the brain of a calf with Sporadic Bovine Encephalomyelitis [33] whereas *C. pecorum* IPA originated from a joint of a polyarthritic sheep.[35] Both strains were able to establish systemic infections as shown in previous studies [46,47] and also confirmed in this study.

Histologically, most severe joint lesions were observed in the IPA i.v. group with a predominantly chronic inflammatory type (lymphoplasmahistiocytic cell infiltrates and granulation tissue). Chlamydiae are able to persist in their host triggering ongoing inflammatory responses resulting in chronicity. [5] The intravenously inoculated E58 strain resulted in mild histological joint lesions while the same strain inoculated intraarticularly resulted in severe lesions. For the IPA strain, both inoculation routes resulted in moderate to severe lesions. Altogether, the results suggest that the IPA isolate is more virulent than E58 in this challenge model. Previous potential explanations for this difference [22] include that, (i) the IPA strain was originally isolated from a sheep with polyarthritis and is better adapted to this host (ii) the conversion of reticulate to elementary bodies *in vitro* is six hours earlier in IPA than E58 possibly resulting in more replication competence [21], and (iii) previous studies demonstrated pathogenetic diversity caused by minor genomic variations among different *Chlamydia* strains.[39,27] Moreover, previous *in vivo* and *in vitro* investigations [21,25] indicated that the *C. pecorum* IPA isolate is a plasmid-bearing strain while E58 is not and a direct relationship between the presence of a plasmid and the strain

469 virulence is strongly suspected. Chlamydial release via host cell lysis appears to be
470 plasmid regulated [53] and results in more infectious EBs available to infect the
471 neighbouring cells.

472 In naturally infected sheep (n=5), the majority of joint lesions were chronic-active
473 inflammatory lesions (lymphoplasmahistiocytic and purulent arthritis/synovitis) (4/5
474 animals), while the majority of joints of experimentally infected animals showed
475 chronic inflammatory lesions without active components (lymphoplasmahistiocytic
476 including granulation tissue). Although experimentally and naturally infected animals
477 were in the same age range, the duration of the *C. pecorum* infection in naturally
478 infected animals is unknown preventing a direct comparison.
479 Immunohistochemistry (IHC) detected chlamydial antigen in the carpal joint of animal
480 628 from the IPA i.a. group, in the stifle joint of animal 658 from the IPA i.v. group
481 and in carpal, hock and stifle joint of animal 710 from E58 i.a. group. Chlamydial
482 inclusions were found in macrophages and occasional in synovial fibroblasts and
483 synoviocytes. Similar to these investigations, other studies described epithelial or
484 epithelial-like cells as primary chlamydial host cells. [12] Also synoviocytes have been
485 found to be suitable host cells for *C. trachomatis* [20] and *C. pecorum* isolates can
486 infect circulating monocytes/macrophages.[21,14] This, and the detection of *C. pecorum*
487 DNA in blood and organs, the detection of IgG and IgM antibodies in sera of infected
488 animals and the isolation of live organisms from joints of i.v. infected animals as
489 described [22] leads to the assumption, that there is systemic, hematogenous
490 spreading of *C. pecorum,* possibly with the help of macrophages.

491 Chlamydial antigen was rarely detected by IHC in joint tissues and tissues of inner
492 organs of experimentally infected animals, whereas the corresponding qPCRs were
493 positive in joints (53.44%) and inner organs (40.83%). In naturally infected animals,

chlamydial antigen was detected in 5/5 animals by IHC in joint tissues and tissues of inner organs, whereas the corresponding qPCRs were positive in 4/5 joint tissues from three animals. A low amount of antigen, visible in only single positive cells could indicate a low level or subclinical infection which could explain the low levels of IHC positivity.[9] Furthermore, IHC only detects inclusions while PCR also detects individual elementary and reticulate bodies as well as providing an estimate of DNA copies and is therefore the more sensitive method. Moreover, sections taken from various levels of a FFPE block are unable to be compared directly with PCR and IHC investigations.[6]

In this study, chlamydial IHC results have been included in our newly developed semi-quantitative scoring system for the assessment of histomorphological joint lesions. The first step towards the development of our scoring system was the identification of previously established scoring systems.[34,29,16] After the designation of inflammatory features, it was important to avoid extreme ranges of severity levels [42] to create an adaptive, reproducible and easily applicable scoring system which started with an unblinded initial evaluation, followed by a targeted masked review.[31] The inflammatory features have been determined (i) on the basis of standard definitions for chronic/active inflammations under the supervision of a dipl. ECVP pathologist and (ii) based on the comparison of synovial pathology features with normal synovial tissue as previously assessed for human rheumatology research.[10,15] Interestingly, calculation of the mean scores of all groups revealed the highest score in stifle joints, followed by carpal and hock joints, although the administration route in the i.a. group was the carpal joint. The reasons for the stifle joint as a predilection site remains unclear but might be the result of rapid systemic spread of the infection.

519 To our knowledge, a thorough description of *C. pecorum*-induced histopathological
520 lesions in inner organs of sheep has not been performed until now. Histologically,
521 severe lesions in experimentally infected animals were observed in the kidneys,
522 most severe in the IPA i.v. group. The kidneys showed a chronic or chronic-active
523 pyelitis, which partially ascended into the interstitium. As seen in the IPA i.v. group,
524 in 5/5 animals of naturally infected animals, a chronic (animals number 3619 and
525 7629) or chronic-active (animals number 8452, 7578 and 8084) pyelitis, which
526 partially ascended into the interstitium, was seen. In koalas, a thorough investigation
527 of organ distribution and histopathological findings due to chlamydial infection was
528 performed and 6/23 koalas showed pan-glomerular sclerosis, mild
529 glomerulonephritis and also chronic interstitial nephritis with severe chronic-active
530 pyelitis.[9] Another study revealed, that genetically distinct *C. pecorum* strains have
531 distinct tissue tropisms in the koala and that plasmid bearing *C. pecorum* strains in
532 the upper genital tract correlates with urogenital disease, suggesting that plasmids
533 expand the genetic diversity of the infecting *Chlamydia*, and can increase
534 virulence.[36] Thus, there are similarities between kidney lesions in koalas and sheep
535 due to an infection with *C. pecorum*.

536 Interestingly, 4/20 infected sheep of both groups (IPA i.a. (n=1), IPA i.v. (n=2), E58
537 i.v. (n=2) showed a mild chronic lymphohistiocytic (meningo-) encephalitis which
538 have been to our knowledge only reported in calves due to *C. pecorum* and dogs
539 due to the chlamydial agent of ovine polyarthritis, strains LW 679 and LW 38 until
540 now.[48,49,54]
541 In experimentally infected animals, immunohistochemical labeling to detect Iba1
542 (macrophages), CD20 (B cells) and CD3 (T cells) antigens was histologically most
543 obvious for detecting macrophages, followed by B cells and T cells with the highest

544 presence in carpal joints followed by stifle and hock joints. In naturally infected
545 animals, immunohistochemical labeling to detect macrophages, B and T cells, was
546 also most obvious for detecting macrophages. Macrophages as well as monocytes
547 and mucosal epithelial cells of respiratory, gastrointestinal, urogenital tract or
548 conjunctival epithelium as well as trophoblastic epithelium of the placenta are well
549 known as chlamydial target cells.[44,32,38] In human medicine, previous observations
550 indicated, that monocytic cells constitute the primary host cell type for *C.*
551 *trachomatis*-induced reactive arthritis in synovial tissue.[11] Former research groups
552 detected *C. trachomatis* inclusions within blood monocytes via transmisson electron
553 microscopy and chlamydial major outer membrane protein via immunoelectron
554 microscopy.[30]
555
556 **Conclusions**
557 The objective of the present study was to investigate histologic changes due to *C.*
558 *pecorum* in tissues of carpal, hock and stifle joints as well as spleen, liver, kidney,
559 lymph nodes and brain of 35 sheep. The most obvious inflammatory lesions were
560 observed in joints and the renal pelvis from experimentally, especially in the IPA-
561 infected group, and naturally infected animals. The immunohistochemical
562 assessment of presence and distribution of macrophages, T and B cells in joints
563 revealed the highest presence of macrophages. Former observations, that *C.*
564 *pecorum* infected circulating monocytes and our findings of histological lesions in
565 joints and inner organs with the detection of *C. pecorum* DNA, underlines the
566 assumption of a systemic, hematogenous spread of this agent. In human medicine,
567 monocytic cells constitute the primary host cell type for *C. trachomatis*-induced
568 reactive arthritis in synovial tissue. The immunohistochemical assessment, reported

here, with the highest presence of macrophages has led to the assumption that monocytic cells also constitute the primary host cell type for *C. pecorum.* This is a valuable finding for further investigations regarding the pathogenesis of *C. pecorum* and the development of a vaccine in order to minimize the consequences of chlamydial polyarthritis and the economic losses due to *C. pecorum* infection.

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The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Figure legends

Figure 1. Qualitative assessment of histological joint lesions. Assessment of the presence of inflammation, the type of inflammation, the degree and distribution of inflammation and whether the surrounding tissue is affected or not.

Figure 2. Qualitative assessment of histological lesions of inner organs. Identification of four main microscopic findings, focussing on inflammation and the qualitative assessment of the presence of inflammation, the type, degree and distribution of inflammation.

Figure 3-10. Histologic findings and additional synovial findings in joints of experimentally and naturally *C. pecorum* infected sheep. Hematoxylin and eosin (HE). **Figure 3.** Chronic arthritis. Carpal joint, experimentally infected animal number 328, E58 i.a. group. 100x. HE. Insert: moderate chronic multifocal lymphoplasmahistiocytic arthritis, lymphocytes (arrow) plasma cells (arrow), histiocytes (arrow). 400x. HE. **Figure 4**. Chronic arthritis with granulation tissue. Carpal joint, experimentally infected animal number 723, IPA i.a. group. Moderate chronic multifocal lymphoplasmahistiocytic arthritis with granulation tissue (arrows). 100x. HE. **Figure 5**. Chronic-active arthritis. Carpal joint, experimentally infected animal number 673, carpus, E58 i.a. group. 100x. HE. Severe chronic-active multifocal lymphohistiocytic and purulent arthritis and synovitis with fibrin accumulations (arrow). Insert: neutrophils (arrow) and fibrin (arrow). 400x. HE. **Figure 6.** Chronic-active arthritis with fibrin accumulations. Carpal joint, experimentally infected animal number 710, E58 i.a. group. 100x. HE. Severe

chronic-active multifocal lymphohistiocytic arthritis with fibrin accumulations (arrows). Insert: plasma cells (arrow), histiocytes (arrow) and neutrophils (arrow). 400x. HE. **Figure 7.** Additional synovial findings. Carpal joint, experimentally infected animal number 628, IPA i.a. group. Areolar folding (arrows). 100x. HE. **Figure 8.** Additional synovial findings. Stifle joint, experimentally infected animal number 694, E58 i.v. group. Neovascularization (arrows) with areolar folding convoluted into vascularisated villi. 100x. HE. **Figure 9.** Active arthritis. Stifle joint, naturally infected animal number 3619. 100x. HE. Mild to moderate active multifocal purulent synovitis. Insert: neutrophils (arrows). 400x. HE. **Figure 10.** Chronic arthritis. Carpal joint, naturally infected animal number 8452. 100x. HE. Moderate to severe chronic multifocal plasmacellular arthritis. Insert: plasma cells (arrows). 400x. HE.

Figure 11-18. Histologic findings in inner organs of experimentally and naturally *C. pecorum* infected sheep. Hematoxylin and eosin (HE). **Figure 11.** Chronic meningoencephalitis. Brain, experimentally infected animal number 658, IPA i.v. group. Moderate chronic multifocal lymphohistiocytic meningoencephalitis (arrows). 100x. HE. Insert: focal glia nodule. 400x. HE. **Figure 12.** BALT hyperplasia. Lung, experimentally infected animal number 616, IPA i.v. group. 100x. HE. **Figure 13.** Secondary follicles. Lymph node, experimentally infected animal number 710, E58 i.a. group. 100x. HE. **Figure 14.** Chronic periportal hepatitis. Liver, experimentally infected animal number 591, E58 i.v. group. Mild to moderate chronic lymphoplasmacellular periportal hepatitis. 100x. HE. Insert: leukocytostasis (arrows). 400x. HE. **Figure 15**. Chronic-active pyelitis. Kidney, experimentally infected animal number 531, E58 i.v. group. Severe chronic-active multifocal purulent and lymphohistiocytic pyelitis (arrows), ascending into the papilla. 100x. HE. Insert: lymphocytes (arrow), macrophages (arrow), neutrophil (arrow). 400x. HE. **Figure 16.**

Chronic-active pyelitis. Kidney, experimentally infected animal number 616, IPA i.v. group. Severe chronic-active multifocal purulent and lymphoplasmahistiocytic pyelitis with follicle formation (arrows). 100x. HE. **Figure 17.** Chronic pyelitis. Kidney, naturally infected animal number 3619. Moderate to severe chronic multifocal lymphoplasmahistiocytic pyelitis. 100x. HE. Insert: lymphocytes (arrow), plasma cells (arrow), macrophages (arrow). 400x. HE. **Figure 18.** Chronic-active conjunctivitis. Conjunctiva, naturally infected animal number 3619. Severe chronic-active multifocal lymphoplasmacellular and purulent conjunctivitis with follicle formation (arrow). 100x. HE. Insert: plasma cells (arrow), neutrophils (arrow). 400x. HE.

Figure 19-22. Immunohistochemistry for chlamydial labeling (Chlc) in joints and inner organs of experimentally and naturally *C. pecorum* infected sheep. **Figure 19.** Positive immunohistochemical labeling for chlamydial antigen (Chlc). Carpal joint, experimentally infected animal number 628, IPA i.a. group. 200x. Chlc. Insert: positive granular intracytoplasmic labeling, mostly in macrophages (arrows). 400x. Chlc. **Figure 20.** Positive immunohistochemical labeling for chlamydial antigen (Chlc). Kidney, experimentally infected animal number 616, IPA i.v. group. 200x. Chlc. Insert: positive granular intracytoplasmic labeling in the lining epithelium of the renal papilla (arrow). 400x. Chlc. **Figure 21.** Positive immunohistochemical labeling for chlamydial antigen (Chlc). Carpal joint, naturally infected animal number 3619. 200x. Chlc. Insert: positive granular intracytoplasmic labeling, mostly in macrophages (arrows). 400x. Chlc. **Figure 22.** Positive immunohistochemical labeling for chlamydial antigen (Chlc). Kidney, naturally infected animal number 7578. 200x. Chlc. Insert: single positive cell underneath the epithelium of the renal papilla (arrow). 400x. Chlc.

Figure 23-28. Immunohistochemistry for detecting macrophages (Iba1), B cells (CD20) and T cells (CD3) in joints of experimentally and naturally *C. pecorum* infected sheep. **Figure 23.** Immunohistochemistry for detecting macrophages (Iba1). Carpal joint, experimentally infected animal number 723, IPA i.a. group. 100x. Iba1. Insert: positive immunohistochemical labeling for detecting Iba 1 antigen. 400x. Iba1. **Figure 24.** Immunohistochemistry for dectecting B cells (CD20). Carpal joint, experimentally infected animal number 628, IPA i.a. group. 100x. CD20. Insert: positive immunohistochemical labeling for detecting CD20 antigen. 400x. CD20. **Figure 25.** Immunohistochemistry for dectecting T cells (CD3). Carpal joint, experimentally infected animal number 673, E58 i.a. group. 100x. CD3. Insert: positive immunohistochemical labeling for detecting CD3 antigen. 400x. CD3. **Figure 26.** Immunohistochemistry for detecting macrophages (Iba1). Carpal joint, naturally infected animal number 7578. 200x. Iba1. Insert: positive immunohistochemical labeling for detecting Iba 1 antigen. 400x. Iba1. **Figure 27.** Immunohistochemistry for detecting B cells (CD20). Carpal joint, naturally infected animal number 7629. 200x. CD20. Insert: positive immunohistochemical labeling for detecting CD20 antigen. 400x. CD20. **Figure 28.** Immunohistochemistry for detecting T cells (CD3). Carpal joint, experimentally infected animal number 8452. 200x. CD3. Insert: positive immunohistochemical labeling for detecting CD3 antigen. 400x. CD3.

Figure 29. Mosaicplot, CD3 labeling, experimentally infected animals. The groups IPA i.a., IPA i.v. and E58 i.a. differed significantly from the control group with the highest presence in carpal joints followed by stifle and hock joints (evaluated using mixed models).

Figure 30. Mosaicplot, CD20 labeling, experimentally infected animals. CD20 labeling revealed a significant difference in the E58 i.a., IPA i.a. and IPA i.v. group when compared to the control group and with the highest presence in carpal joints followed by stifle and hock joints (evaluated using mixed models).

Figure 31. Mosaicplot, Iba1 labeling, experimentally infected animals. For Iba1 labeling, the groups E58 i.a., IPA i.v. and IPA i.a. group differed significantly from the control group with the highest presence in carpal joints followed by stifle and hock joints (evaluated using mixed models).

Annexes

Table 1: Euthanasia was performed at various time points after the administration of group-specific inoculum.

group	animal ID	days after inoculation until euthanasia
control	356	22
	373	22
	351	22
	655	22
	687	15
UV i.v.[a]	393	15
	681	21
	685	18
	671	21
	604	21
UV i.a.[b]	396	21
	568	18
	595	21
	394	21
	544	15
IPA i.v.[c]	335	21
	658	15
	320	9
	591	21
	616	18
IPA i.a.[d]	628	21
	661	10
	578	10
	723	9
	716	18
E 58 i.v.[e]	365	21
	694	15

	593	18
	339	4
	531	21
E58 i.a.[f]	370	15
	328	18
	710	9
	315	4
	673	21

[a]*C. pecorum,* UV- irradiated. Route of administration: intravenous (i.v.)

[b]*C.pecorum,* UV- irradiated. Route of administration: intraarticular (i.a.)

[c]*C. pecorum, strain IPA.* Route of administration: intravenous (i.v.)

[d]*C. pecorum,* strain IPA. Route of administration: intraarticular (i.a.)

[e]*C. pecorum*, strain E58. Route of administration: intravenous (i.v.)

[f]*C. pecorum,* strain E58. Route of administration: intraarticular (i.a.)

Table 2: Semiquantitative parameters (scoring system) for the histological examination of joint lesions.

para-meters		score	0	1	2	3	maximal score per joint	maximal score per animal
inflammation	acute	**numbers of foci per section**	0	1-5	6-10	>10	3	9
		distribution		focal	multifocal	diffuse		
		degree	npf[a]	mild[b]	moderate[c]	severe[d]	3	9
	chronic	**numbers of foci per section**	0	1-5	6-10	>10	3	9
		distribution		focal	multifocal	diffuse		
		degree	npf[a]	mild[b]	moderate[c]	severe[d]	3	9
other parameters	**presence of fibrin**		no	yes	n/a[e]	n/a[e]	1	3
	presence of granulation tissue		no	yes	n/a[e]	n/a[e]	1	3

surrounding tissue affected	no	yes	n/a[e]	n/a[e]	1	3
IHC positive	no	yes	n/a[e]	n/a[e]	1	3
maximal total score					**16**	**48**

[a]npf = no pathological findings

[b]mild = low number of inflammatory cells

[c]moderate = moderate number of inflammatory cells

[d]severe = severe number of inflammatory cells

[e]n/a= analysis was not applicable

Table 3: Additional histological findings in joints of experimentally infected animals.

	animal/ group						
parameter	**control**	**UV, i.a.[a]**	**UV, i.v.[b]**	**E58, i.a.[c]**	**E58, i.v.[d]**	**IPA, i.a.[e]**	**IPA, i.v.[f]**
synoviocyte proliferation - palisading - areolar folding	0/5	1/5	0/5	5/5	4/5	5/5	5/5
neovascularisation - areolar folding convoluted into vascularisated villi	0/5	0/5	0/5	4/5	2/5	3/5	5/5
periarterial fibrosis	0/5	0/5	0/5	2/5	1/5	2/5	4/5
total score/ group	**0**	**1**	**0**	**11**	**7**	**10**	**14**

[a] *C. pecorum*, UV- irradiated. Route of administration: intraarticular (i.a.)

[b] *C. pecorum*, UV- irradiated. Route of administration: intravenous (i.v.)

[c] *C. pecorum,* strain E58. Route of administration: intraarticular (i.a.)

[d] *C. pecorum*, strain E58. Route of administration: intravenous (i.v.)

[e] *C. pecorum,* strain IPA. Route of administration: intraarticular (i.a.)

[f] *C. pecorum, strain IPA*. Route of administration: intravenous (i.v.)

Table 4: Semiquantitative parameters (scoring system) for the histological examination of inner organs (brain, kidney, liver).

parameters		score	0	1	2	3	maximal score per organ	maximal score per animal
inflammation	acute	**numbers of foci per section**	**0**	**1-5**	**6-10**	**>10**	3	9
		distribution		focal	multifocal	diffuse		
		degree	npf[a]	mild[b]	moderate[c]	severe[d]	3	9
	chronic	**numbers of foci per section**	**0**	**1-5**	**6-10**	**>10**	3	9
		distribution		focal	multifocal	diffuse		
		degree	npf[a]	mild[b]	moderate[c]	severe[d]	3	9
IHC positive		n/a[e]	no	yes	n/a[e]	n/a[e]	1	3
maximal total score							**13**	**39**

npf= no pathological findings

[b]mild = low number of inflammatory cells

[c]moderate = moderate number of inflammatory cells

[d]severe = severe number of inflammatory cells

[e]n/a= analysis was not applicable

Table 5: Antibodies used for immunophenotyping of macrophages, B- and T- lymphocytes in joints.

	primary antibody	epitope demasking	dilution	incubation	secondary antibody
CD3	DAKO, M7254	Dkb[a] (pH9, 98°C, 20 min)	1:50	1h	EnV.Ms[b], substrate DAB
CD20	Thermo Scientific, RB9013-P	without	1:100	30min	EnV.Rb[c], substrate DAB
Iba 1	WAKO, 019-19741	Dkc[d] (pH6, 98°C, 20 min)	1:350	1h	EnV.Rb[c], substrate DAB

[a]basic buffer heat

[b]Envision mouse antibody

[c]Envision rabbit antibody

[d]citrat buffer heat

Table 6: Histopathologic joint diagnoses in experimentally infected animals grouped according to inoculum, route of administration, immunohistochemistry, total semiquantitative score per group and additional findings.

group	chronic inflammation[a]	chronic-active/ active inflammation[b]	score	ChIc[c]	additional findings[d]
control	1/15[e]	0/15	6	negative	0
UV- IPA, i.a.[f]	2/15	1/15[g]	10	negative	1
UV- IPA, i.v.[h]	2/15	0/15[i]	8	negative	0
E58, i.a.[j]	5/15	7/15	74	positive (1/5)	11
E58, i.v.[k]	3/15	2/15	18	negative	7
IPA, i.a.[l]	1/15	5/15	66	positive (1/5)	10
IPA, i.v.[m]	8/15	5/15	79	positive (1/5)	14

[a]number of joints per group with chronic arthritis/ synovitis. In total: fifteen joints per group (carpal, hock and stifle joint per animal)

[b] number of joints per group with chronic- active or active arthritis/ synovitis. In total: fifteen joints per group (carpal, hock and stifle joint per animal)

[c]IHC to detect chlamydial antigen

[d] additional findings: score per group

[e]one case: acute periarthritis, possibly injection- site induced, not included in scoring

[f]*C.pecorum,* UV- irradiated. Route of administration: intraarticular (i.a.)

[g]one case: acute focal fibrositis, not included in scoring

[h]*C. pecorum,* UV- irradiated. Route of administration: intravenous (i.v.)

[i]one case: acute focal cellulitis and hemorrhage, not included in scoring

[j]*C. pecorum,* strain E58. Route of administration: intraarticular (i.a.)

[k]*C. pecorum*, strain E58. Route of administration: intravenous (i.v.)

[l]*C. pecorum,* strain IPA. Route of administration: intraarticular (i.a.)

[m]*C. pecorum, strain IPA.* Route of administration: intravenous (i.v.)

Table 7: Histopathologic diagnoses of inner organs (brain, kidney, liver) of experimentally infected animals grouped according to inoculum, route of administration, immunohistochemistry, total semiquantitative score per group and additional findings.

group	organ	chronic inflammation[a]	active inflammation[b]	score	Chlc[c]
control	brain	0/5[d]	0/5	6	negative
	kidney	0/5	0/5		
	liver	1/5[e]	1/5[f]		
UV- IPA, i.a.[g]	brain	1/5[h]	0/5	12	negative
	kidney	0/5	0/5		
	liver	3/5	1/5[f]		
UV- IPA, i.v.[i]	brain	0/5[d]	0/5	6	negative
	kidney	0/5	0/5		
	liver	1/5	1/5[f]		
E58, i.a.[j]	brain	0/5	0/5	26	negative
	kidney	3/5[k,l]	1/5		
	liver	3/5	0/5		
E58, i.v.[m]	brain	2/5[d]	0/5	32	negative
	kidney	0/5	2/5[f]		

	liver	3/5	3/5		
IPA, i.a.[n]	brain	1/5	0/5	15	positive (1/5)
	kidney	2/5	0/5		
	liver	1/5	3/5		
IPA, i.v.[o]	brain	2/5	0/5	60	positive (2/5)
	kidney	1/5[k]	3/5[f]		
	liver	1/5	3/5		

[a]number of inner organs per group with chronic inflammation.

[b]number of inner organs per group with chronic-active or active inflammation.

[c]Immunohistochemistry to detect Chlamydial antigen.

[d]1/5= mild and focal chronic encephalitis, not included in scoring.

[e]1/5= mild and focal chronic periportal hepatitis, not included in scoring.

[f]leukocytostasis.

[g]*C.pecorum,* UV- irradiated. Route of administration: intraarticular (i.a.).

[h]focal glia nodule, negative tested for toxoplasma antigen.

[i]*C. pecorum,* UV- irradiated. Route of administration: intravenous (i.v.).

[j]*C. pecorum,* strain E58. Route of administration: intraarticular (i.a.).

[k]also interstitial nephritis.

[l]1/5= mild chronic and focal lymphocytic pyelitis, not included in scoring.

[m]*C. pecorum*, strain E58. Route of administration: intravenous (i.v.).

[n]*C. pecorum,* strain IPA. Route of administration: intraarticular (i.a.).

[o] *C. pecorum, strain IPA.* Route of administration: intravenous (i.v.).

Figure 1:

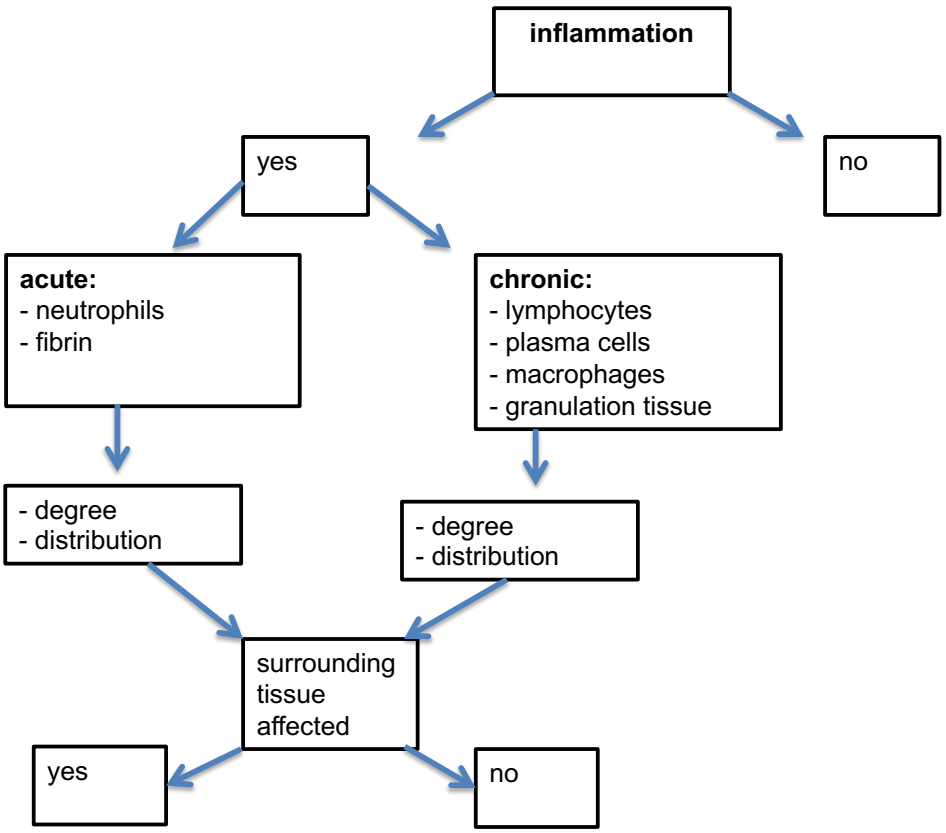

inflammation
yes
no
acute:
- neutrophils
- fibrin
chronic:
- lymphocytes
- plasma cells
- macrophages
- granulation tissue
- degree
- distribution
- degree
- distribution
surrounding tissue affected
yes
no

Figure 2:

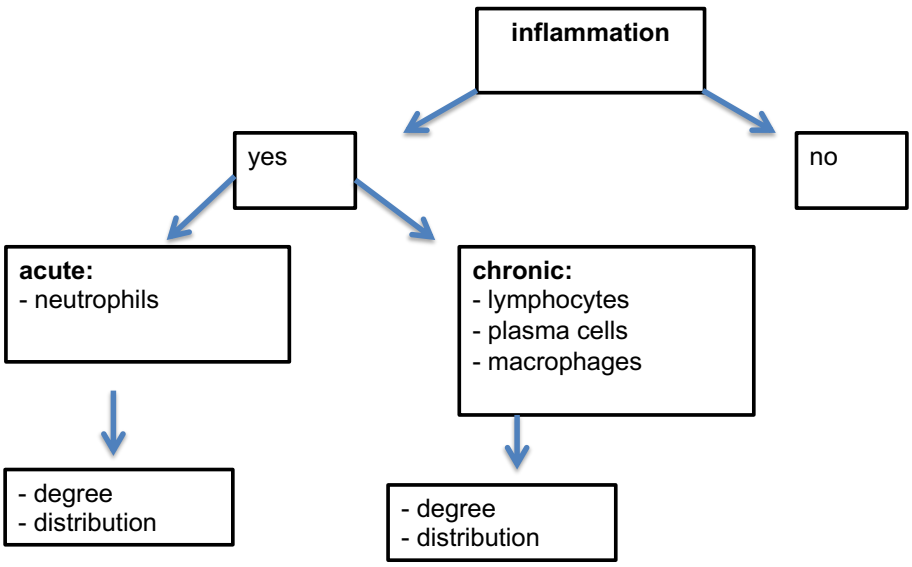

inflammation
yes
no
acute:
- neutrophils
chronic:
- lymphocytes
- plasma cells
- macrophages
- degree
- distribution
- degree
- distribution

Figure 3-10:

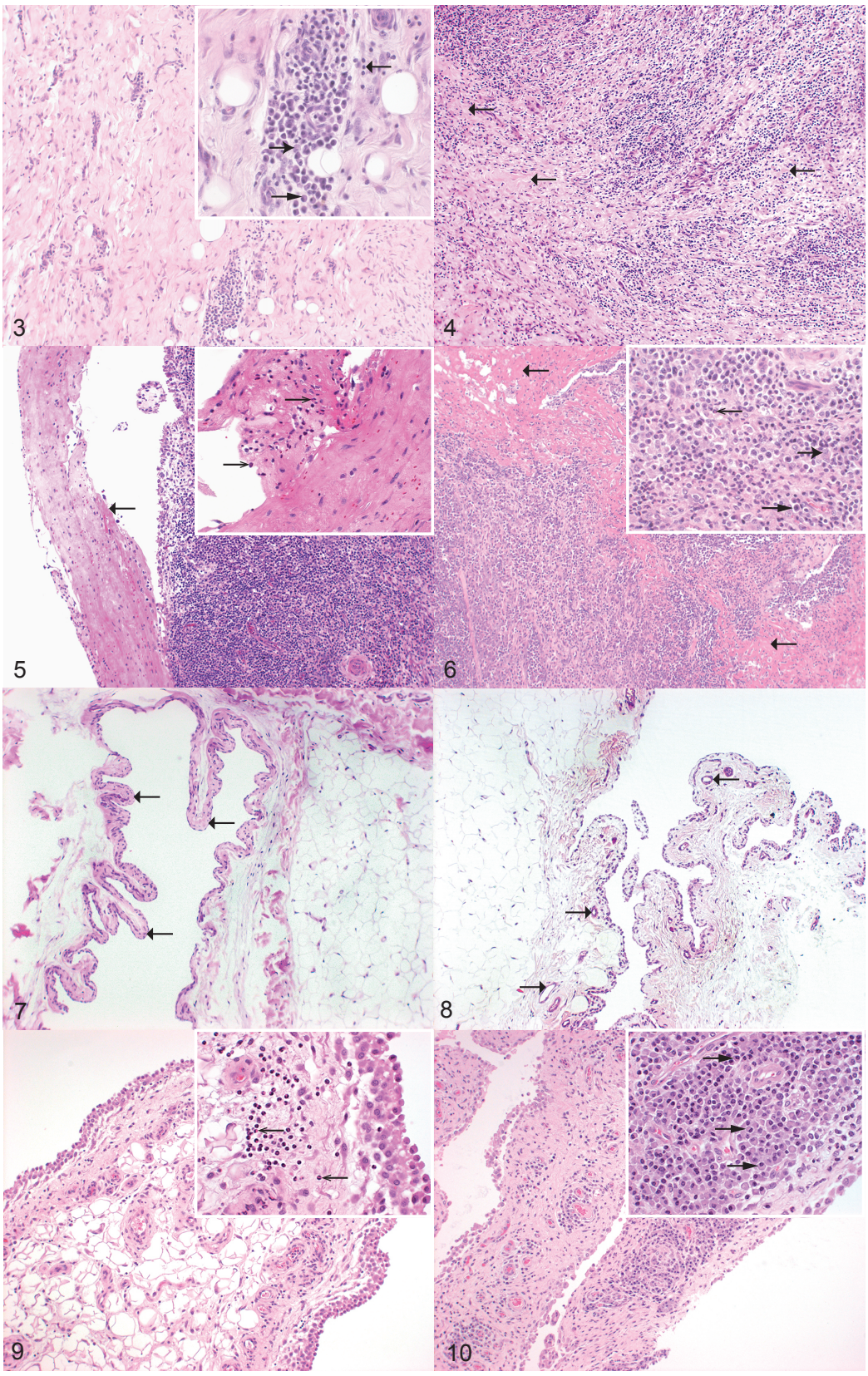


Figure 11-18:

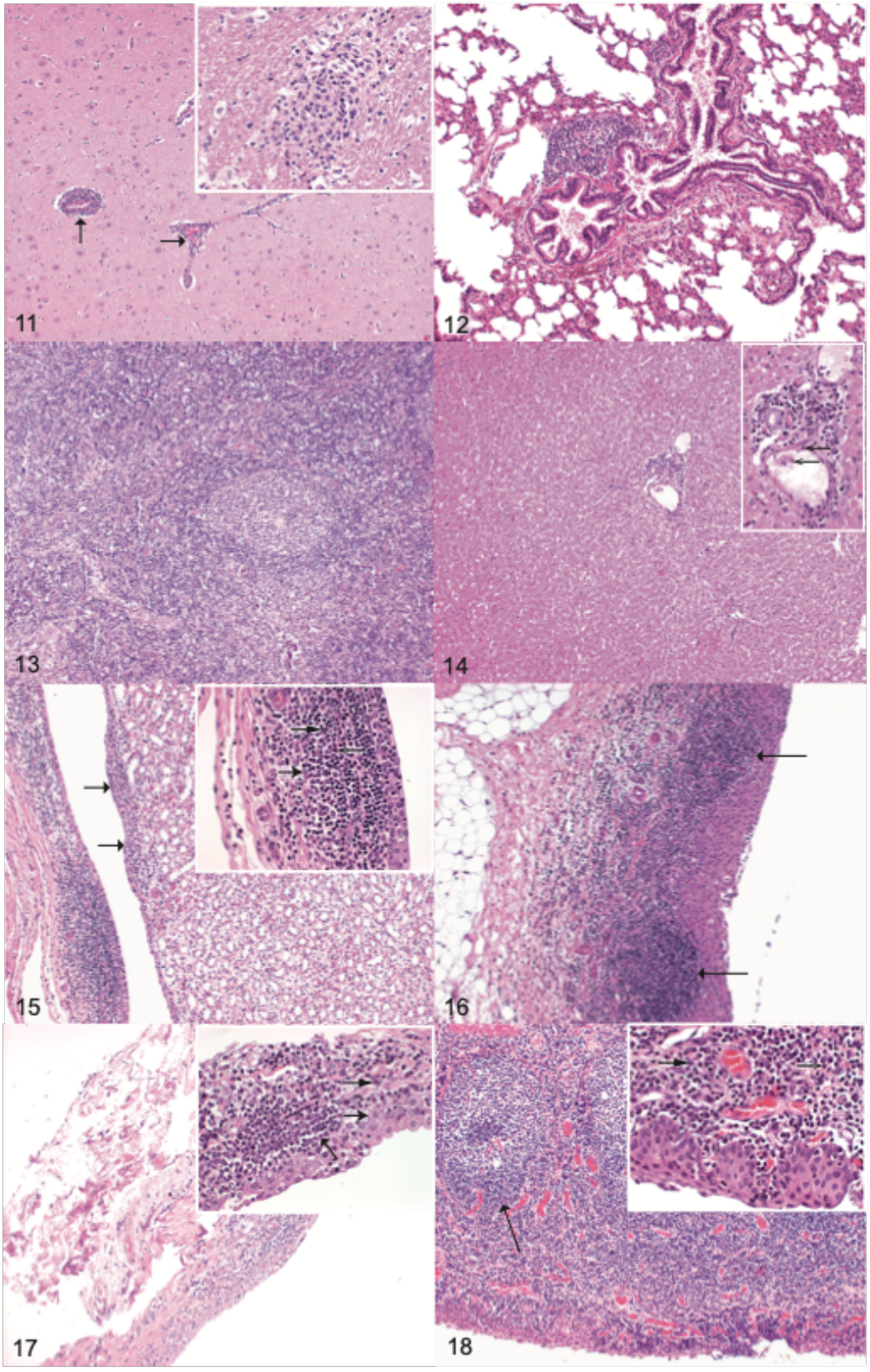


Figure 19-22:

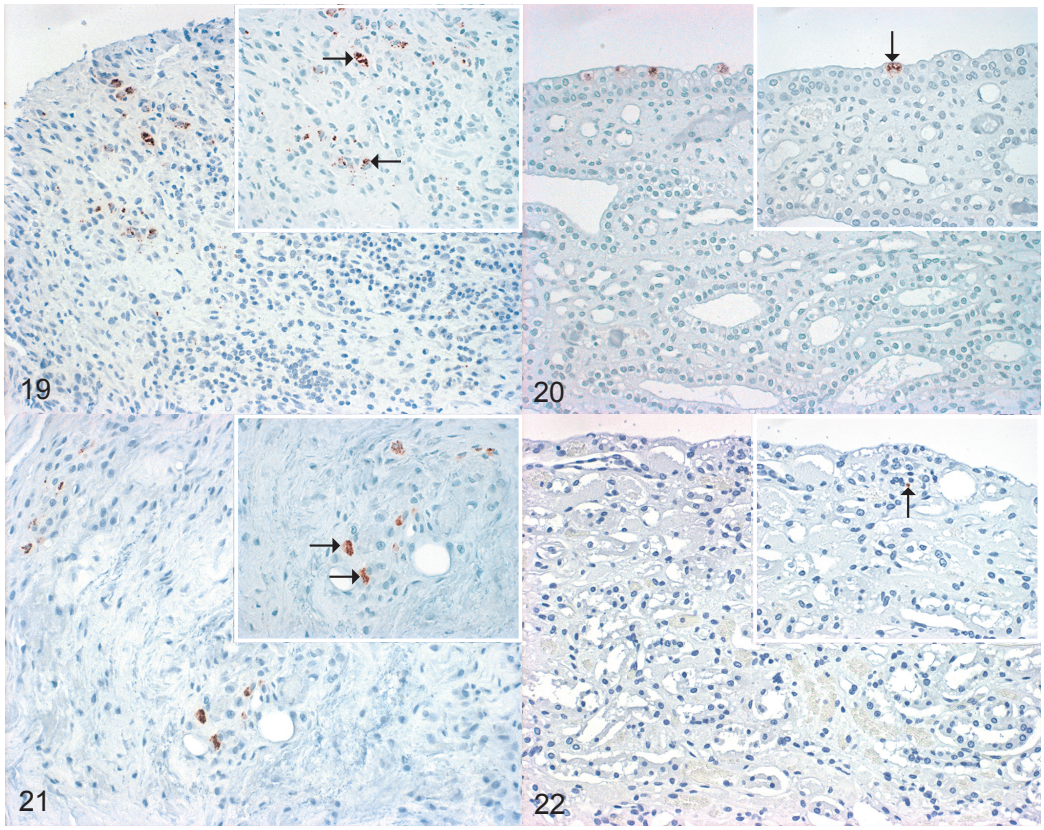


Figure 23-28:

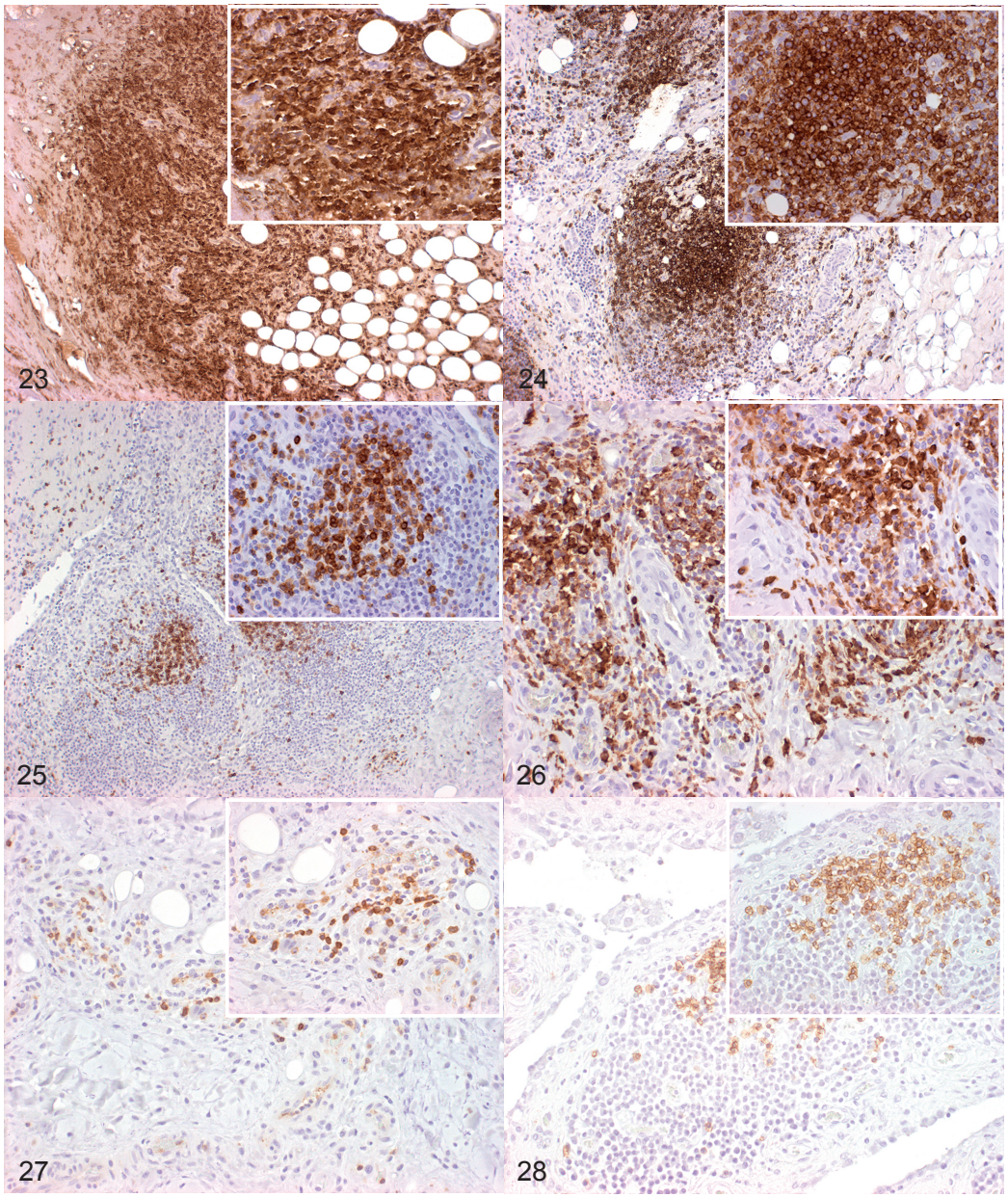


Figure 29:

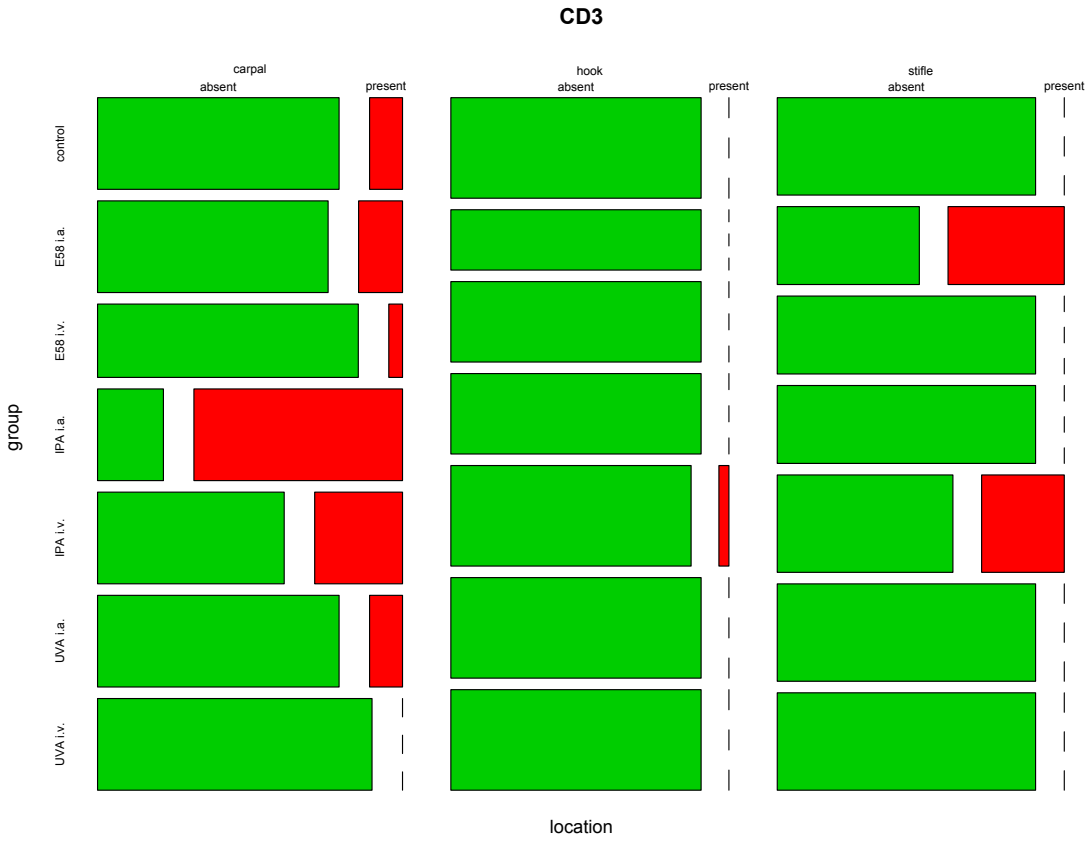

CD3
carpal
absent
present
hook
absent
present
stifle
absent
present
control
E58 i.a.
E58 i.v.
IPA i.a.
IPA i.v.
UVA i.a.
UVA i.v.
group
location

Figure 30:

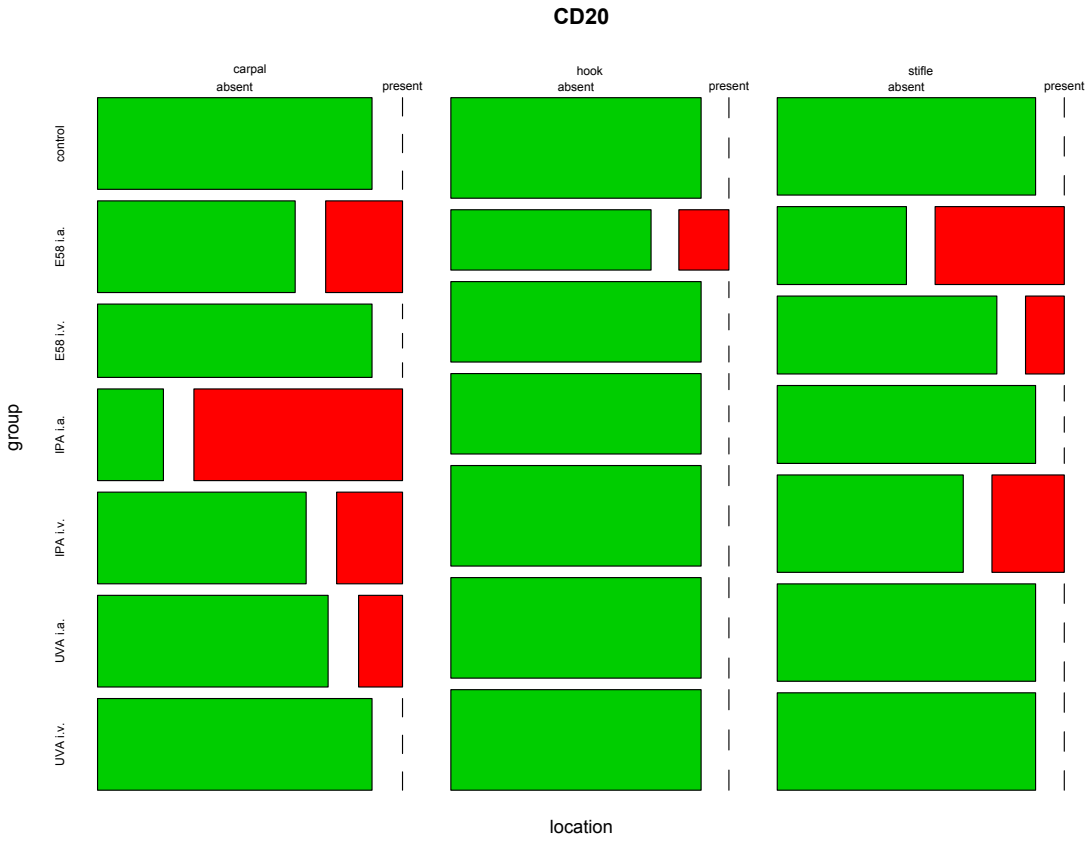
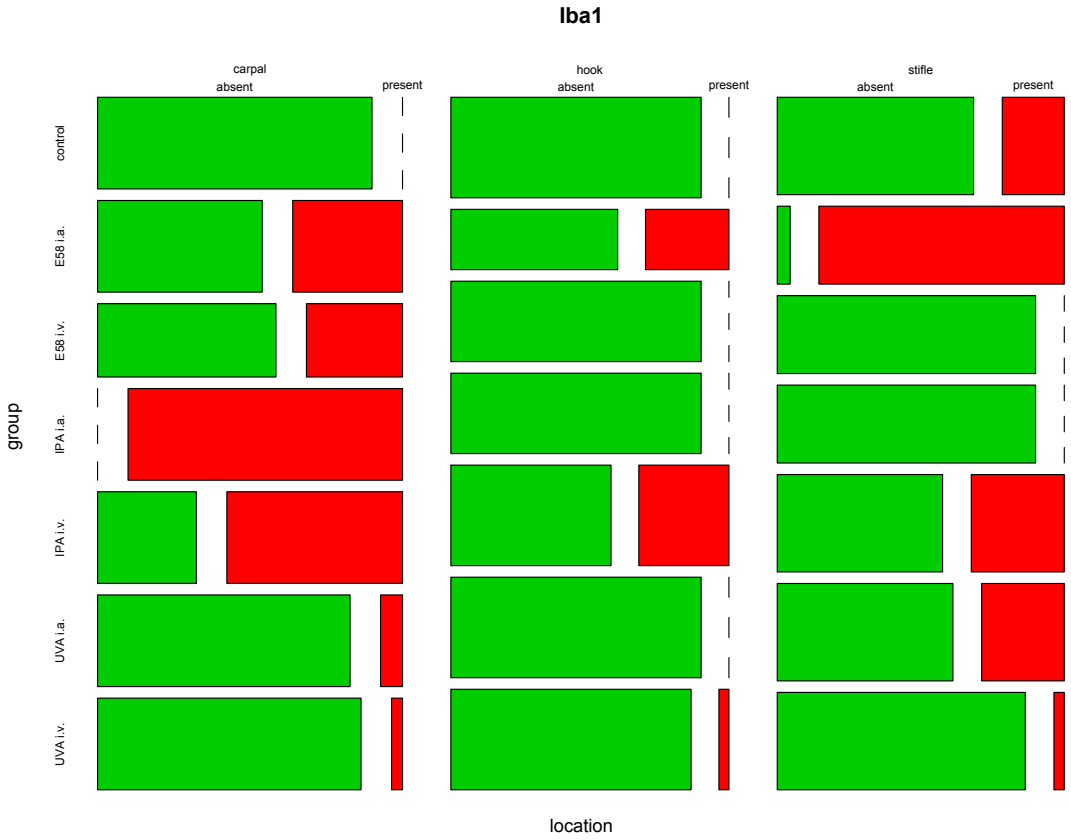

CD20
carpal
absent
present
hook
absent
present
stifle
absent
present
control
E58 i.a.
E58 i.v.
IPA i.a.
IPA i.v.
UVA i.a.
UVA i.v.
group
location

Figure 31:


Iba1
carpal
absent
present
hook
absent
present
stifle
absent
present
control
E58 i.a.
E58 i.v.
IPA i.a.
IPA i.v.
UVA i.a.
UVA i.v.
group
location

Supplementary Table 1: Histopathologic joint diagnoses from experimentally *C. pecorum*- infected animals grouped according to inoculum, route of administration, qPCR, immunohistochemistry (IHC, ChIC), and total score per animal.

group	animal ID / joint	administration route	pathological- anatomical diagnosis (HE)[a]	qPCR (copies/µl)	IHC (ChIC)[b]	total score/ animal
control	351/ carpal	i.a.[c]	npf[d]	0	neg[e]	0
	351/ hock			0		
	351/ stifle			0		
	655/ carpal		npf[d]	0	neg[e]	3
	655/ hock			0		
	655/ stifle		moderate chronic focal lymphohistiocytic peri-arthritis[f] (possibly injection site- induced)	0		
	356/ carpal		npf[d]	0	neg[e]	0
	356/ hock			0		
	356/ stifle			0		
	687/ carpal		moderate chronic focal lymphohistiocytic synovitis[g]	0	neg[e]	3
	687/ hock		npf[d]	0		
	687/ stifle			0		
	373/ carpal		npf[d]	0	neg[e]	0
	373/ hock			0		
	373/ stifle			0		
***C. pecorum* UV- IPA[h]**	396/ carpal	i.a.[c]	npf[d]	0	neg[e]	0
	396/ hock			0		
	396/ stifle			0		
	568/ carpal		npf[d]	0	neg[e]	1
	568/ hock		mild acute focal purulent fibrositis	0		
	568/ stifle		npf[d]	0		
	595/ carpal		npf[d]	0	neg[e]	0
	595/ hock			0		
	595/ stifle			0		
	394/ carpal		npf[d]	0	neg[e]	2
	394/ hock			0		
	394/ stifle		mild chronic multifocal lymphoplasmahistiocytic synovitis[g]	0		
	544/ carpal		mild chronic multifocal lymphohistiocytic synovitis[g]	0	neg[e]	7
	544/ hock		npf[d]	0		
	544/ stifle		moderate chronic- active multifocal lymphoplasmahistiocytic and purulent arthritis[i] and synovitis[g]	0		
	393/ carpal	i.v.[j]	npf[d]	0	neg[e]	4
	393/ hock		mild acute focal purulent cellulitis with mild focal haemorrhage	0		
	393/ stifle		npf[d]	0		
	671/ carpal		npf[d]	0	neg[e]	0
	671/ hock			0		
	671/ stifle			0		
	681/ carpal		npf[d]	0	neg[e]	2
	681/ hock		mild chronic multifocal lymphocytic synovitis[g]	0		
	681/ stifle		npf[d]	0		

	685/ carpal		mild chronic multifocal lymphohistiocytic synovitis[g]	0	neg[e]	2
	685/ hock		npf[d]	0		
	685/ stifle			0		
	604/ carpal		npf[d]	0	neg[e]	0
	604/ hock			0		
	604/ stifle			0		
***C. pecorum* IPA[k]**	716/ carpal	i.a.[c]	npf[d]	113278	neg[e]	12
	716/ hock			0		
	716/ stifle		severe chronic- active multifocal lymphoplasmahistiocytic and purulent arthritis[i]	0		
	628/ carpal		severe chronic- active multifocal lymphoplasmahistiocytic and purulent arthritis[i]	24564	pos[l]	12
	628/ hock		npf[d]	0	neg[e]	
	628/ stifle			0		
	661/ carpal		npf[d]	178342	neg[e]	13
	661/ hock			0		
	661/ stifle		severe chronic- active multifocal lymphoplasmahistiocytic and purulent arthritis[i] and synovitis[g]	71		
	578/ carpal		moderate chronic- active multifocal lymphoplasmacellular and purulent arthritis[i] and synovitis[g]	83619	neg[e]	23
	578/ hock		tissues missing	0		
	578/ stifle		moderate chronic- active multifocal lymphoplasmacellular and purulent arthritis[i] and synovitis[g]	0		
	723/ carpal		moderate chronic multifocal lymphoplasmacellular arthritis[i] and synovitis[g]	142313	neg[e]	6
	723/ hock		tissues missing	3532		
	723/ stifle			3067		
	658/ carpal	i.v.[j]	mild chronic- active diffuse lymphoplasmahistiocytic arthritis[i]	8192	neg[e]	24
	658/ hock		npf[d]	361		
	658/ stifle		severe chronic- active lymphocytic and purulent diffuse arthritis[i]	2342	pos[l]	
	616/ carpal		moderate chronic diffuse lymphocytic arthritis[i] with mild acute focal purulent synovitis[g]	0	neg[e]	21
	616/ hock		mild chronic- active lymphocytic and purulent focal synovitis[g]	347		
	616/ stifle		mild chronic focal lymphoplasmacellular arthritis[i] with mild chronic-active focal lymphoplasmacellular and purulent synovitis[g]	467		
	335/ carpal		moderate chronic multifocal lymphohistiocytic arthritis[i]	0	neg[e]	14
	335/ hock		moderate chronic multifocal lymphohisticytic arthritis[i] and synovitis[g]	365		
	335/ stifle		tissues missing	524		
	320/ carpal		mild chronic multifocal lymphoplasmahistiocytic arthritis[i] and synovitis[g]	0	neg[e]	14
	320/ hock		tissues missing	130		
	320/ stifle		mild chronic multifocal lymphohisticytic synovitis[g]	130		
	591/ carpal		npf[d]	2506	neg[e]	6
	591/ hock		moderate chronic multifocal lymphocytic arthritis[i] and synovitis[g]	190		
	591/ stifle		mild chronic multifocal lymphoplasmacellular synovitis[g]	0		
***C. pecorum* E58[m]**	370/ carpal	i.a.[c]	npf[d]	9641	neg[e]	18

	370/ hock		moderate chronic multifocal lymphohistiocytic arthritis[i] with severe chronic- active lymphohistiocytic and purulent synovitis[g]	0		
	370/ stifle		mild chronic- active focal lymphohistiocytic and purulent synovitis[g]	0		
	710/ carpal		severe chronic- active multifocal lymphoplasmahistiocytic and purulent arthritis[i] and moderate acute multifocal purulent synovitis[g]	71303	pos[l]	25
	710/ hock		tissues missing	0		
	710/ stifle		severe chronic- active multifocal lymphohistiocytic and purulent synovitis[g]	0		
	315/ carpal		severe chronic multifocal lymphoplasmahistiocytic arthritis[i] and moderate acute multifocal purulent synovitis[g]	124059	neg[e]	11
	315/ hock		tissues missing	0		
	315/ stifle			0		
	673/ carpal		severe chronic multifocal lymphoplasmahistiocytic arthritis[i] and synovitis[g]	167349	neg[e]	7
	673/ hock		npf[d]	0	neg[e]	
	673/ stifle			0		
	328/ carpal		severe chronic multifocal lymphoplasmahistiocytic arthritis[i] moderate acute multifocal purulent synovitis[g]	87848	neg[e]	13
	328/ hock		npf[d]	0		
	328/ stifle		moderate chronic focal lymphohistiocytic arthritis[i]	0		
	365/ carpal	i.v.[j]	npf[d]	0	neg[e]	3
	365/ hock			92		
	365/ stifle		mild chronic multifocal lymphoplasmahistiocytic synovitis[g]	0		
	593/ carpal		moderate chronic multifocal lymphohistiocytic synovitis[g]	1884	neg[e]	7
	593/ hock		npf[d]	421		
	593/ stifle		mild acute focal purulent synovitis[g]	0		
	339/ carpal		tissues missing	0	neg[e]	n/a
	339/ hock			337		
	339/ stifle			419		
	694/ carpal		npf[d]	251	neg[e]	8
	694/ hock			0		
	694/ stifle		mild to moderate acute multifocal purulent and mild chronic focal lymphohistiocytic arthritis[i]	347		
	531/ carpal		npf[d]	0	neg[e]	0
	531/ hock			488		
	531/ stifle			0		

[a]HE = hematoxylin and eosin staining.
[b]IHC = immunohistochemistry for chlamydial antigen (ChlC).
[c]i.a. = intraarticular (administration in carpal joint of right front leg).
[d]npf = no pathological findings.
[e]neg = negative.
[f]periarthritis: area around joint inflamed.
[g]synovitis: only synovia inflamed.
[h]*C.pecorum*, UV- irradiated.
[i]arthritis: joint inflamed.
[j]i.v. = intravenous (administration in jugular vein).
[k]*C. pecorum*, strain IPA.
[l]pos= positive.
[m]*C. pecorum*, strain E58.

Supplementary Table 2: Histopathologic diagnoses of joints and inner organs from naturally *C. pecorum*- infected animals, qPCR, chlamydial immunohistochemistry (IHC, ChlC), and total score per animal.

animal ID/ organ	Histopathologic diagnosis (HE)[a]	qPCR (copies/µl)	IHC (ChlC)[b]	score/ animal
3619, lung	npf[c]	n/a[d]	negative	**51**
3619, liver	mild chronic focal lymphocytic periportal hepatitis			
3619, spleen	npf[c]			
3619, kidney	moderate to severe chronic multifocal lymphoplasmacellular pyelitis, mildly ascending into the papilla			
3619, lymphnode	npf[c]			
3619, conjunctiva	severe chronic- active multifocal lymphoplasmacellular and purulent conjunctivitis with follicle formation			
3619, left carpal joint	moderate to severe chronic multifocal lymphoplasmacellular arthritis and chronic-active multifocal lymphoplasmacellular and purulent synovitis (mainly perivascular)	BDL[e]	positive	
3619, right carpal joint	moderate to severe chronic multifocal lymphoplasmacellular arthritis and chronic-active multifocal lymphoplasmacellular and purulent synovitis (mainly perivascular)	9.31	positive	
3619, right stifle joint	mild to moderate active multifocal purulent synovitis and mild chronic multifocal lymphoplasmacytic arthritis	163	negative	
8452, lung	npf[c]	n/a[d]	negative	**32**
8452, liver	mild chronic multifocal lymphocytic periportal hepatitis			
8452, spleen	npf[c]			
8452, kidney	npf[c]		positive	
8452, lymphnode	npf[c]		negative	
8452, left carpal joint	moderate to severe chronic multifocal plasmacellular arthritis and chronic-active multifocal plasmacellular and purulent synovitis (mainly perivascular)	BDL[e]	negative	
8452, right carpal joint	moderate chronic multifocal lymphoplasmahistiocytic arthritis and synovitis (esp. perivascular) and mild acute multifocal purulent synovitis	BDL[e]		
7578, lung	npf[c]	n/a[d]	negative	**30**
7578, liver	mild chronic multifocal lymphocytic periportal hepatitis			
7578, spleen	npf[c]			
7578, kidney	mild chronic-active focal lymphocytic and purulent pyelitis		positive	
7578, lymphnode	npf[c]		negative	
7578, left carpal joint	mild chronic multifocal lymphohistiocytic arthritis	34		
7578, right carpal joint	mild to moderate chronic multifocal lymphohistiocytic arthritis	BDL[e]	positive	
7629, lung	npf[c]	n/a[d]	negative	**15**
7629, liver	mild to moderate chronic multifocal lymphocytic periportal hepatitis			
7629, spleen	npf[c]			
7629, kidney	mild chronic multifocal lymphocytic pyelitis			
7629, lymphnode	npf[c]			
7629, left carpal joint	mild to moderate chronic-active multifocal purulent and lymphocytic arthritis and mild chronic multifocal synovitis	6.76	positive	

7629, right carpal joint	npf[c]	BDL[e]	negative	
8084, lung	npf[c]	n/a[d]	negative	**27**
8084, liver	mild chronic multifocal lymphocytic periportal hepatitis			
8084, kidney	moderate to severe chronic-active multifocal lymphohistiocytic and purulent pyelitis, mildly ascending into the papilla			
8084, lymphnode	npf[c]			
8084, left carpal joint	mild to moderate chronic-active multifocal lymphohistiocytic and purulent arthritis and synovitis	BDL[e]		
8084, right carpal joint	mild to moderate chronic multifocal lymphoplasmahistiocytic arthritis	BDL[e]	positive	

[a]HE = hematoxylin and eosin staining.
[b]IHC = immunohistochemistry for chlamydial antigen (ChlC).
[c]no pathologic findings.
[d]n/a= analysis was not applicable.
[e]BDL=below detection limit, but HRM (= High Resolution Melting Analysis) was present.

Supplementary Table 3: Histopathologic diagnoses of inner organs from experimentally *C. pecorum*- infected animals per group, route of administration, qPCR, chlamydial immunohistochemistry (IHC, ChlC), and total score per animal.

group	animal ID / organ	administration route	histopathologic diagnosis (HE)[a]	qPCR (copies/µl)	IHC (ChlC)[b]	score/ animal
control	351/ lung	i.a.[c]	npf[d]	0	neg[e]	4
	351/ lnn			0		
	351/ brain		mild chronic focal lymphohistiocytic encephalitis (perivascular)	0		
	351/ kidney		npf[d]	0		
	351/ spleen			0		
	351/ liver		mild chronic multifocal lymphocytic periportal hepatitis	0		
	655/ lung		npf[d]	0	neg[e]	1
	655/ lnn			0		
	655/ brain			0		
	655/ kidney			0		
	655/ spleen			0		
	655/ liver		mild focal chronic lymphocytic periportal hepatitis	0		
	356/ lungs		npf[d]	0	neg[e]	0
	356/ lnn			0		
	356/ brain			0		
	356/ kidney			0		
	356/ spleen			0		
	356/ liver			0		
	687/ lungs		npf[d]	0	neg[e]	1
	687/ lnn			0		
	687/ brain			0		
	687/ kidney			0		
	687/ spleen			0		
	687/ liver		leukocytostasis	0		
	373/ lungs		npf[d]	0	neg[e]	0
	373/ lnn			0		
	373/ brain			0		
	373/ kidney			0		
	373/ spleen			0		
	373/ liver			0		
UV-IPA[f]	396/ lung	i.a.[c]	npf[d]	0	neg[e]	2
	396/ lnn			0		
	396/ brain			0		
	396/ kidney			0		
	396/ spleen			0		
	396/ liver		mild multifocal chronic lymphocytic periportal hepatitis	0		
	568/ lung		npf[d]	0	neg[e]	1
	568/ lnn			0		
	568/ brain			0		

	568/ kidney			0		
	568/ spleen			0		
	568/ liver		leukocytostasis	0		
	595/ lung		npf[d]	0	neg[e]	2
	595/ lnn			0		
	595/ brain			0		
	595/ kidney			0		
	595/ spleen			0		
	595/ liver		mild chronic multifocal lymphocytic periportal hepatitis	0		
	394/ lung		npf[d]	0	neg[e]	4
	394/ lnn			0		
	394/ brain		mild chronic multifocal histiocytic encephalitis (perivascular)	0		
	394/ kidney		npf[d]	0		
	394/ spleen			0		
	394/ liver		mild chronic multifocal lymphoplasmahistiocytic periportal hepatitis moderate multifocal hepatolipidosis	0		
	544/ lung		npf[d]	0	neg[e]	1
	544/ lnn		tissues missing	0		
	544/ brain		focal glia nodule	0		
	544/ kidney		mild chronic focal lymphocytic pyelitis	0		
	544/ spleen		npf[d]	0		
	544/ liver			0		
UVA- IPA[f]	393/ lung	i.v.[g]	npf[d]	0	neg[e]	1
	393/ lnn			0		
	393/ brain		mild chronic focal lymphocytic encephalitis (perivascular)	0		
	393/ kidney		npf[d]	0		
	393/ spleen			0		
	393/ liver			0		
	671/ lung		npf[d]	0	neg[e]	0
	671/ lnn			0		
	671/ brain			0		
	671/ kidney			0		
	671/ spleen			0		
	671/ liver			0		
	681/ lung		npf[d]	0	neg[e]	0
	681/ lnn			0		
	681/ brain			0		
	681/ kidney			0		
	681/ spleen			0		
	681/ liver			0		
	685/ lung		npf[d]	0	neg[e]	3
	685/ lnn			0		
	685/ brain			0		
	685/ kidney			0		
	685/ spleen			0		
	685/ liver		mild chronic- active multifocal neutrophilic and lymphocytic interstitial hepatitis	0		

			leucocytostasis			
	604/ lung		npf[d]	0	neg[e]	2
	604/ lnn			0		
	604/ brain			0		
	604/ kidney			0		
	604/ spleen			0		
	604/ liver		mild chronic multifocal lymphocytic periportal hepatitis	0		
***C. pecorum* IPA[h]**	716/ lung	i.a.[c]	npf[d]	0	neg[e]	6
	716/ lnn			0		
	716/ brain			0		
	716/ kidney		mild chronic multifocal lymphohistiocytic pyelitis	0		
	716/ spleen		npf[d]	137		
	716/ liver		mild chronic multifocal lymphocytic periportal hepatitis leukocytostasis	193		
	628/ lung		npf[d]	212	neg[e]	3
	628/ lnn			0		
	628/ brain			0		
	628/ kidney			0		
	628/ spleen			0		
	628/ liver		mild acute multifocal neutrophilic periportal hepatitis	0		
	661/ lung		npf	0	neg[e]	3
	661/ lnn			315		
	661/ brain			0		
	661/ kidney		mild chronic multifocal lymphocytic pyelitis	158		
	661/ spleen		npf	0		
	661/ liver		leukocytostasis	0		
	578/ lung		npf	0	neg[e]	0
	578/ lnn			315		
	578/ brain		mild chronic multifocal lymphohistiocytic meningoencephalitis	877		
	578/ kidney		npf[d]	0		
	578/ spleen			0		
	578/ liver			0		
	723/ lung		npf[d]	0	neg[e]	0
	723/ lnn			614		
	723/ brain			0		
	723/ kidney		npf[d] , renal pelvis partially missing	0		
	723/ spleen		npf[d]	0		
	723/ liver			0		
***C. pecorum* IPA[h]**	658/ lung	i.v.[g]	npf[d]	168	neg[e]	16
	658/ lnn			912		
	658/ brain		moderate chronic multifocal lymphohistiocytic meningoencephalitis focal glia nodule	387		
	658/ kidney		severe chronic-active multifocal purulent and lymphoplasmahistiocytic pyelitis, slightly ascending into the papilla mild chronic- active multifocal perivascular lymphocytic and purulent nephritis with fibrosis	266	neg[e]	
	658/ spleen		npf[d]	778	neg[e]	

	658/ liver		mild hepatolipidosis leukocytostasis	2344		
	616/ lung		npf[d]	0	neg[e]	17
	616/ lnn			2087		
	616/ brain			652		
	616/ kidney		severe chronic- active multifocal purulent and lymphoplasmahistiocytic pyelitis with follicle formation, ascending to the papilla moderate chronic- active focal lymphohistiocytic and purulent interstitial nephritis (neutrophils in the tubules- lumina, tubules are activated and partially degenerated)	170	focal: positive	
	616/ spleen		npf[d]	0	neg[e]	
	616/ liver		mild chronic multifocal lymphocytic periportal hepatitis mild multifocal hepatolipidosis	716		
	335/ lung		npf[d]	0	neg[e]	12
	335/ lnn			694		
	335/ brain			0		
	335/ kidney		severe chronic- active multifocal purulent and lymphohistiocytic pyelitis with follicle formation, ascending into the papilla moderate chronic focal lymphocytic interstitial nephritis	865		
	355/ spleen		npf[d]	622		
	335/ liver			0		
	320/ lung		npf[d]	0	neg[e]	3
	320/ lnn			362		
	320/ brain			0		
	320/ kidney		mild chronic multifocal lymphocytic pyelitis	0		
	320/ spleen		npf[d]	276		
	320/ liver		leuocytostasis mild multifocal hepatolipidosis	245		
	591/ lung		npf[d]	0	neg[e]	6
	591/ lnn			0		
	591/ brain		mild chronic multifocal lymphohistiocytic meningoencephalitis	350		
	591/ kidney		npf[d]	347		
	591/ spleen			0		
	591/ liver		leukocytostasis mild to moderate chronic multifocal lymphoplasmacellular periportal hepatitis	103		
***C. pecorum* E 58[i]**	328/ lung	i.a.[c]	npf	681	neg[e]	2
	328/ lnn			0		
	328/ brain			882		
	328/ kidney		mild chronic focal lymphocytic interstitial nephritis (renal pelvis partially missing)	0		
	328/ spleen		npf[d]	0		
	328/ liver			610		
	370/ lung		npf[d]	200	neg[e]	2
	370/ lnn			0		
	370/ brain			0		
	370/ kidney		mild chronic multifokal lymphoplasmacellular pyelitis	671		
	370/ spleen		npf[d]	0		

	370/ liver			116		
	710/ lung		npf[d]	0	neg[e]	3
	710/ lnn			0		
	710/ brain			0		
	710/ kidney		mild chronic focal lymphohistiocytic interstitial nephritis	0		
	710/ spleen		npf[d]	0		
	710/ liver		mild chronic multifocal lymphohistiocytic periportal hepatitis	0		
	315/ lung		npf[d]	0	neg[e]	15
	315/ lnn			0		
	315/ brain			0		
	315/ kidney		severe chronic- active multifocal purulent and lymphoplasmahistiocytic pyelitis	0		
	315/ spleen		npf[d]	147		
	315/ liver		mild chronic multifocal lymphocytic periportal hepatitis	0		
	673/ lung		severe acute diffuse congestion	0	neg[e]	3
	673/ lnn		npf[d]	1045		
	673/ brain			341		
	673/ kidney		additional findings: mild multifocal tubular calcification, hyperemia missing renal pelvis	0		
	673/ spleen		npf[d]	0		
	673/ liver		mild chronic multifocal lymphocytic periportal hepatitis	0		
***C. pecorum* E 58[i]**	694/ lung	i.v.[g]	npf[d]	116	neg[e]	4
	694/ lnn			0		
	694/ brain			0		
	694/ kidney			0		
	694/ spleen			0		
	694/ liver		mild chronic multifocal lymphohistiocytic periportal hepatitis leukocytostasis	2136		
	365/ lung		npf[d]	232	neg[e]	4
	365/ lnn			137		
	365/ brain		mild chronic multifocal lymphohistiocytic meningoencephalitis glia nodes	918		
	365/ kidney		renal pelvis missing	0		
	365/ spleen		npf[d]	0		
	365/ liver		mild chronic multifocal lymphocytic periportal hepatitis leukocytostasis	721		
	593/ lung		npf[d]	0	neg[e]	0
	593/ lnn			0		
	593/ brain			565		
	593/ kidney			0		
	593/ spleen			803		
	593/ liver			0		
	339/ lung		npf[d]	0	neg[e]	3
	339/ lnn			0		
	339/ brain			825		
	339/ kidney		mild active multifocal purulent pyelitis	0		
	339/ spleen		npf[d]	0		

	339/ liver			0		
	531/ lung		npf[d]	773	neg[e]	18
	531/ lnn			733		
	531/ brain		mild chronic multifocal lymphohistiocytic meningitis	681		
	531/ kidney		severe chronic- active multifocal purulent and lymphohistiocytic urethritis and pyelitis, ascending into the papilla	936		
	531/ spleen		npf[d]	0		
	531/ liver		mild chronic lymphohistiocytic multifocal periportal hepatitis leukocytostasis	0		

[a]HE = hematoxylin and eosin staining.
[b]IHC = immunohistochemistry for chlamydial antigen (ChlC).
[c]i.a. = intraarticular (administration in carpal joint of right front leg).
[d]npf = no pathological findings.
[e]neg = negative.
[f]*C.pecorum,* UV- irradiated.
[g]i.v. = intravenous (administration in jugular vein).
[h]*C. pecorum,* strain IPA.
[i]*C. pecorum*, strain E58.

Supplementary Table 4: results of the descriptive analysis (mean/median/sd) (standard derivation) of joints (carpal, hock, stifle) and inner organs (kidney, brain, lung, lymphnodes, spleen) of experimentally infected animals including qPCR data.

score_joints	**carpal, median/mean/sd:** 0.00/ 3.23/ 4.50		**hock, median/mean/sd:** 0.00/ 1.35/ 2.93			**stifle, median/mean/sd:** 1.00/ 3.34/ 4.51	
score_ joints per group	**control median/mean/sd:** 0.00/ 0.40/ 1.06	**UVA i.a. median/mean/sd:** 0.00/ 0.53/ 1.36	**UVA i.v. median/mean/sd:** 0.00/ 0.53/ 1.19	**E58 i.a. median/mean/sd:** 6.00/ 6.17/ 5.51	**E58 i.v. median/mean/sd:** 0.00/ 1.50/ 2.54	**IPA i.a. median/mean/sd:** 0.00/ 5.08/ 5.94	**IPA i.v. median/mean/sd:** 6.00/ 5.27/ 3.71
score_per organ	**brain:** 0.00/ 0.66/ 1.21			**kidney:** 0.00/ 2.17/ 4.25			
score organs (brain+ kidney) per group	**control** 0.00/ 0.20/ 0.63	**UVA i.a.** 0.00/ 0.20/ 0.63	**UVA i.v.:** 0.00/ 0.10/ 0.31	**E58 i.a.:** 0.00/ 1.70/ 3.71	**E58 i.v.** 0.00/ 0.10/ 3.75	**IPA i.a.** 0.00/ 0.80/ 1.32	**IPA i.v.** 2.50/ 4.80/ 5.37
lung: score_peribronchial accumulation per group	**control** 0.00/1.40/1.52	**UVA i.a.** 0.00/1.80/ 1.30	**UVA i.v.** 0.00/ 1.20/ 2.17	**E58 i.a.:** 0.00/ 0.60/ 0.89	**E58 i.v.** 0.00/ 0.60/ 0.89	**IPA i.a.** 0.00/ 2.20/ 2.28	**IPA i.v.** 0.00/ 2.00/ 0.70
lung: score_peribronchial lymphfollicles per group	**control** 3.00/ 3.60/ 1.95	**UVA i.a.** 5.00/ 5.20/ 2.05	**UVA i.v.** 3.00/ 3.60/ 1.82	**E58 i.a.** 3.00/ 3.20/ 1.64	**E58 i.v.** 3.00/ 3.80/ 2.77	**IPA i.a.** 2.00/ 3.00/ 1.41	**IPA i.v.** 5.00/ 5.20/ 1.30
lymphnode: score_follicles per group	**control** 0.01/ 0.01/ 0.01	**UVA i.a.** 0.02/ 0.02/ 0.01	**UVA i.v.** 0.02/ 0.02/ 0.01	**E58 i.a.** 0.01/ 0.01/ 0.01	**E58 i.v.** 0.03/ 0.02/ 0.01	**IPA i.a.** 0.01/ 0.01/ 0.01	**IPA i.v.** 0.01/ 0.01/ 0.01
lymphnodes: score_secondary follicles per group	**control** 0.02/ 0.02/ 0.01	**UVA i.a.** 0.01/ 0.02/ 0.01	**UVA i.v.** 0.02/ 0.02/ 0.01	**E58 i.a.** 0.01/ 0.01/ 0.01	**E58 i.v.** 0.03/ 0.03/ 0.01	**IPA i.a.** 0.01/ 0.01/ 0.01	**IPA i.v.** 0.01/ 0.01/ 0.02
spleen: score_follicles per group	**control** 0.01/ 0.02/ 0.02	**UVA i.a.** 0.01/ 0.02/ 0.01	**UVA i.v.** 0.01/ 0.01/ 0.01	**E58 i.a.** 0.01/ 0.01/ 0.00	**E58 i.v.** 0.01/ 0.01/ 0.01	**IPA i.a.** 0.01/ 0.02/ 0.01	**IPA i.v.** 0.01/ 0.01/ 0.01
spleen: score_secondary follicles per group	**control** 0.01/ 0.01/ 0.02	**UVA i.a.** 0.02/ 0.01/ 0.01	**UVA i.v.** 0.01/ 0.01/ 0.01	**E58 i.a.** 0.01/ 0.01/ 0.01	**E58 i.v.** 0.02/ 0.02/ 0.01	**IPA i.a.** 0.01/ 0.02/ 0.03	**IPA i.v.** 0.01/ 0.01/ 0.01
joints: qPCR (copies/µl) per joint	**carpal** 8916.50/ 50757.45/ 63795.55			**hock** 46.00/ 313.15/ 777.59		**stifle** 0.00/ 368.35/ 826.80	
joints: qPCR (copies/µl) per group	**E58 i.a.** 0/ 30680.00/ 54884.12	**E58 i.v.** 92/ 282.60/ 481.66		**IPA i.a.** 71/ 36585.73/ 61196.66		**IPA i.v.** 347/ 1036.93/ 2132.94	
load per carpal joint and group	**E58 i.a.** 59020.00/ 92040.00/ 59020.87	**E58 i.v.** 0.00/ 427.00/ 821.71		**IPA i.a.** 113278.00/ 108423.20/ 58541.31		**IPA i.v.** 0.00/ 2139.60/ 3553.15	
load per hock joint and group	**E58 i.a.** 0.00/ 0.00/ 0.00	**E58 i.v.** 337.00/ 267.60/ 211.76		**IPA i.a.** 0.00/ 706.40/1579.56		**IPA i.v.** 347.00/ 278.60/ 110.53	
load per stifle joint and group	**E58 i.a.** 0.00/ 0.00/ 0.00	**E58 i.v.** 0.00/ 153.20/ 211.32		**IPA i.a.** 0.00/ 627.00/ 1364.01		**IPA i.v.** 467.00/ 692.60/ 948.16	
inner organs: qPCR (copies/µl) per organ	**brain** 170.50/ 323.90/ 367.70	**kidney** 0.00/ 170.65/ 302.38	**liver** 0.00/ 359.20/ 688.34	**lymphnode** 68.50/ 360.70/ 534.17	**lung** 0.00/ 119.10/ 224.55	**spleen** 0.00/ 138.15/ 268.70	

inner organs: qPCR (copies/µl) per group	**E58 i.a.** 0.00/ 156.43/ 300.19	**E58 i.v.** 0.00/ 319.20/ 493.89	**IPA i.a.** 0.00/ 94.03/ 203.86	**IPA i.v.** 255.5/ 411.47/574.18
load per brain and group	**E58 i.a.** 0.00/ 244.60/ 385.70	**E58 i.v.** 681.00/ 597.80/ 360.39	**IPA i.a.** 0.00/ 175.40/ 392.21	**IPA i.v.** 350.00/ 277.80/ 279.06
load per kidney and group	**E58 i.a.** 0.00/ 134.20/ 300.08	**E58 i.v.** 0.00/ 187.20/ 418.59	**IPA i.a.** 0.00/ 31.60/ 70.66	**IPA i.v.** 266.00/ 329.60/ 326.00
load per liver and group	**E58 i.a.** 0.00/ 145.20/ 264.64	**E58 i.v.** 0.00/ 571.40/ 928.69	**IPA i.a.** 0.00/ 38.60/ 86.31	**IPA i.v.** 245.00/ 681.60/ 968.86
load per lymphnode and group	**E58 i.a.** 0.00/ 209.00/ 467.34	**E58 i.v.** 0.00/ 174.00/ 318.07	**IPA i.a.** 315.00/ 248.80/ 257.85	**IPA i.v.** 694.00/ 811.00/ 792.37
load per lung and group	**E58 i.a.** 0.00/ 176.20/ 295.18	**E58 i.v.** 116.00/ 224.20/ 321.51	**IPA i.a.** 0.00/ 42.40/ 94.81	**IPA i.v.** 0.00/ 33.60/ 75.13
load per spleen and group	**E58 i.a.** **n/a**	**E58 i.v.** **n/a**	**IPA i.a.** **n/a**	**IPA i.v.** **n/a**

Supplementary Table 5: Statistical results obtained from mixed effect models (effect sizes and 95% confidence intervals, p-values, p- value) of joints (carpal, hock, stifle), inner organs (kidney, brain, lung, lymphnodes, spleen), biomarker and qPCR values. Assessment and comparison of experimental groups compared to the control group.

score_ joints per group	**UVA i.a. confidence interval/ p-value** 0.23 (-1.32 to 1.78)/ 0.10	**UVA i.v. confidence interval/ p-value** 0.30 (-1.24 to 1.83) /0.98	**E58 i.a. confidence interval/ p-value 2.77 (1.48 to 4.07) / 0.001**	**E58 i.v. confidence interval/ p-value** 1.32 (-0.09 to 2.73) / 0.08	**IPA i.a. confidence interval/ p-value 2.60 (1.29 to 3.90 / 0.001**	**IPA i.v. confidence interval/ p-value 2.61 (1.32 to 3.90) / 0.001**
score organs (brain+ kidney) per group	**UVA i.a.** 2.28 (-3.24 to 3.24) / 1.00	**UVA i.v.:** -1.00 (-3.34 to 3.14) / 1.00	**E58 i.a.:** 1.50 (-1.74 to 4.74) / 0.69	**E58 i.v.** 1.90 (-1.34 to 5.14) / 0.46	**IPA i.a.** 6.00 (-2.64 to 3.84) / 0.99	**IPA i.v. 4.69 (1.36 to 7.84 / 0.01**
lung: score_peribronchial accumulation per group	**UVA i.a.** 0.40 (-2.21 to 3.01) / 0.91	**UVA i.v.** -0.20 (-2.81 to 2.4) / 0,91	**E58 i.a.:** -0.80 (-3.41 to 1.81) / 0.91	**E58 i.v.** -0.80 (-3.41 to 1.81) / 0.97	**IPA i.a.** 0.80 (-1.81 to 3.41) / 1.00	**IPA i.v.** 0.60 (-2.01 to 3.21) / 0.10
lung: score_peribronchial lymphfollicles per group	**UVA i.a.** 1.60 (-1.69 to 4.89) / 0.10	**UVA i.v.** -1.10 (-3.29 to 3.29) / 1.00	**E58 i.a.** -4.00 (-3.69 to 2.89) / 0.10	**E58 i.v.** 2.00 (-3.09 to 3.49) / 0.60	**IPA i.a.** -6.00 (-3.89 to 2.69) / 1.00	**IPA i.v.** 1.60 (-1.69 to 4.89) / 0.60
lymphnode: score_follicles per group	**UVA i.a.** 0.01 (-0.01 to 0.03) / 0.34	**UVA i.v.** 0.01 (-0.01 to 0.03) / 0.51	**E58 i.a.** 0.00 (-0.02 to 0.02) / 0.10	**E58 i.v. 0.02 (-0.00 to 0.03) / 0.08**	**IPA i.a.** 0.00 (-0.01 to 0.02) / 0.10	**IPA i.v.** 0.00 (-0.01 to 0.02) / 0.95
lymphnodes: score_secondary follicles per group	**UVA i.a.** -0.00 (-0.02 to 0.02) / 1.00	**UVA i.v.** 0.01 (-0.01 to 0.03) / 0.93	**E58 i.a.** -0.01 (-0.03 to 0.01) / 0.90	**E58 i.v.** 0.01 (-0.01 to 0.03) / 0.60	**IPA i.a.** -0.00 (-0.02 to 0.02) / 1.00	**IPA i.v.** -0.00 (-0.02 to 0.02) / 0.10
spleen: score_follicles per group	**UVA i.a.** -0.00 (-0.02 to 0.01) / 0.99	**UVA i.v.** -0.01 (-0.03 to 0.01) / 0.54	**E58 i.a.** -0.01 (-0.03 to 0.01) / 0.66	**E58 i.v.** -0.01 (-0.02 to 0.01) / 0.78	**IPA i.a.** -0.00 (-0.02 to 0.01) / 0.97	**IPA i.v.** -0.01 (-0.03 to 0.01) / 0.68
spleen: score_secondary follicles per group	**UVA i.a.** 0.00 (-0.03 to 0.03) / 1.00	**UVA i.v.** -0.00 (-0.03 to 0.03) / 1.00	**E58 i.a.** -0.00 (-0.03 to 0.02) / 0.10	**E58 i.v.** 0.01 (-0.02 to 0.04) / 0.10	**IPA i.a.** 0.01 (-0.02 to 0.04) / 0.77	**IPA i.v.** -0.00 (-0.03 to 0.02) / 0.10
score_CD3 per joint	**hock 1.85 (-2.40 to 1.43) / 0.001**			**stifle 3.78 (1.77 to 8.08) / 0.01**		
score_CD3 per group	**UVA i.a.** 1.00 (1.10 to 5.02) / 1.00	**UVA i.v. 1.20 (3.13 to 4.62) / 0.001**	**E58 i.a. 6.33 (1.83 to 2.19) / 0.004**	**E58 i.v.** 3.10 (4.11 to 3.88) / 0.43	**IPA i.a. 1.15 (3.40 to 3.89) /0.001**	**IPA i.v. 8.44 (2.36 to 3.02) / 0.001**
score_CD3 per area	1.0 (8.17 to 1.22) / 1.00					
score_CD20 per joint	**hock 0.06 (0.02 to 0.20) / 0.001**			**stifle 0.48 (0.23 to 0.10) / 0.05**		
score_CD20 per group	**UVA i.a.** 4.27 (0.48 to 37.90) / 0.19	**UVA i.v.** 1.00 (0.06 to 15.64) / 1.00	**E58 i.a. 38.58 (5.35 to 278.26) / 0.001**	**E58 i.v.** 3.98 (0.42 to 37.44) / 0.23	**IPA i.a. 34.49 (4.75 to 250.48) /0.001**	**IPA i.v. 17.30 (2.20 to 135.68) / 0.01**

score_CD20 per area	0.90 (0.73 to 1.10) / 0.30					
score_Iba1 per joint	**hock** **0.16 (0.08 to 0.32) / 0.001**			**stifle** 0.63 (0.33 to 1.20) / 0.16		
score_Iba1 per group	**UVA i.a.** 1.80 (0.69 to 4.76) / 0,23	**UVA i.v.** 0.47 (0.12 to 1.85) / 0.28	**E58 i.a.** **16.27 (6.65 to 39.78) / 0.001**	**E58 i.v.** 1.54 (0.54 to 4.41) / 0.42	**IPA i.a.** **7.76 (3.49 to 17.24) / 0.001**	**IPA i.v.** **11.39 (4.62 to 28.06) / 0.001**
score_Iba1 per area	0.99 (0.85 to 1.34) / 0.83					
load_qPCR per joint: hock/carpal	**hock/ carpal:** **-5.09 (-5.12 to -5.06) / 0.001**			**stifle/ carpal:** **-4.93 (-4.95 to 4.90) / 0.001**		
load_qPCR joint per group:	E58 i.v./ E 58 i.a. **-4.80 (-6.46 to 3.14) / 0.001**	IPA i.a./ E58 i.a. 0.32 (-1.34 to 1.98) / 0.96	IPA i.v./ E58 i.a. **0.32 (-1.4 to 1.98) / 0.001**	IPA i.a. /E58 i.v. **5.12 (3.46 to 6.78) / 0.001**	IPA i.v./ E58 i.v. 0.95 (-0.70 to 2.61) / 0.45	IPA i.v./ IPA i.a. **-4.17 (-5.83 to -2.51) / 0.001**
load_qPCR per organ:	**Kidney** **n/a /** 0.33	**Liver** **n/a /** 0.70	**Lymphnode** **n/a /** 0.52	**Lung** **n/a / 0.04**	**Spleen** **n/a /** 0.26	
load_qPCR per group:	E58 i.v. **n/a /** 0.30	IPA i.a. n/a / 0.10		IPA i.v. n/a / 0.77		

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Curriculum Vitae

Vorname Name	**Nina Ostfeld**
Geburtsdatum	24.02.1988
Geburtsort	Bremen, Deutschland
Nationalität	deutsch
07/2004- 7/2007	**Gymnasium,** Schulzentrum der Sekundarstufe II, Bremen, Deutschland
25.07.2007	**Matura,** Schulzentrum der Sekundarstufe II, Bremen, Deutschland
10/2008- 10/2010	**Ausbildung zur Tiermedizinischen Praxisassistentin,** Tierärztekammer Bremen, Tierarztpraxis Dr. med. vet. Holger Schönert, Bremen, Deutschland
10/2013- 03/2019	**Studium der Tiermedizin**, Justus- Liebig- Universität Giessen, Deutschland
08.02.2019	**Abschlussprüfung vet. med.**, Justus- Liebig- Universität Giessen, Deutschland
04/2019 – 07/2020	**Anfertigung der Dissertation** unter Leitung von Prof. Dr. med. vet. Nicole Borel am Institut für Veterinärpathologie Abteilung für Infektionspathologie der Vetsuisse-Fakultät Universität Zürich Direktorin: Prof. Dr. med. vet., Dipl.ECVP, FRCPath, FTA & FHV (Pathologie) Anja Kipar

02/2019 – 02/2020	Assistenztierärztin, Institut für Veterinärpathologie, Zürich, Schweiz
03/2020 – heute	Assistenztierärztin, Abteilung für Kleintierreproduktion, Tierspital Zürich, Schweiz